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SUSPENSOR AND EARLY EMBRYO OF PINUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 242

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(WITH PLATES VI–X AND THREE FIGURES)

It has long been recognized that the embryos of plants furnish trustworthy morphological features for comparison in the study of phylogeny, but the surprising variations found in the proembryos of various gymnosperms have always been more or less of a stumbling block. This work was undertaken with the hope that a more critical study of the suspensor and early embryo of *Pinus* and of the phenomenon of polyembryony might prove of value in properly interpreting the rather flexible program that has been ascribed to this genus. Here it is, also, that we find a striking parallel to some of the early cleavage phenomena involved in the biology of twins in animals, a subject of some current interest to zoologists.

This paper will limit itself largely to such phases of the embryogeny of *Pinus* as were most effectively studied by means of a special technique for dissection, developed by the writer, and to a discussion of the relation of the early *Pinus* embryo to other conifer types. Certain phases of the later embryo will also be described, but the development of the internal features of the late embryo will be treated in another paper.

Historical

A summary of views in regard to the embryogeny of conifers is given by COULTER and CHAMBERLAIN (**10**), so that it will suffice to note those features in the historical development of the subject which concern our own investigation.

ROBERT BROWN (**2**), in discussing the similarities of the ovulate structures of cycads and conifers, mentioned his own observations of occasional polyembryony in conifers, which was known to be a constant feature in cycads. In a later treatise (**3**) he announced polyembryony as a constant feature among several genera of the Pinaceae and felt convinced that this feature is common to the entire family. He noted the origin of the embryos from "corpuscula" or "areolae," 3–6 in number, at the upper extremity of the "aminos" (endosperm), and pointed out that this provision of several "corpuscula" was like that in cycads, where it also made possible the development of several embryos. He called the suspensors "funiculi," finding that these frequently branch to form still other embryos.

MIRBEL and SPACH (**27**) announced their results from a careful study of several pines and also *Thuja* and *Taxus*, confirming the work of BROWN and extending our knowledge to other forms. In this account these workers were the first to use the terms "suspensor" and "rosette," although in their otherwise excellent figures they show 5 cells in each tier of the early embryo, 5 rosette cells, and 5 vertical rows of cells coming from the base of the corpusculum.

SCHLEIDEN (**36**) gave the first accurate general description of the development of the early embryo, beginning with the "embryonal globule" on the end of the suspensor. His views regarding the earlier stages of the embryo were confused by his erroneous conception that the pollen tube formed the embryo. He pointed out the correct order of appearance of the stem tip meristem and cotyledons, and gave a good account of the formation of the suspensor in its late stages after it becomes massive, describing it as an elongation of cells from the radical portion of the embryo.

HARTIG (**15**) was possibly the first to point out that the upper end of the suspensor is a single cell, but he regarded this cell as

being of vegetative origin. He described a "nest of cells" from which individual cells elongate, and thought that the embryonal tip cell was cut off some time after elongation.

SCHACHT (**35**) agreed with SCHLEIDEN that the pollen tube enters the corpusculum and produces the embryo at its base. He described the rosette correctly as consisting of 4 cells instead of the 5 shown by MIRBEL and SPACH. SCHACHT described the 4 tiers of 4 cells each, known to us as the end product of the proembryo stage. He announced definitely that the 4 rows of cells in *Pinus Pumilio* always separate into 4 embryos and believed that they would split up further. In *Taxus baccata* and *Abies* he reported no splitting of the product of the corpusculum into several embryos.

GOTTSCHE (**14**) gives a critical review and confirmation of the facts known at the time and a more accurate description of the corpusculum, which he found to originate in some unpollinated cycads, and is therefore independent of the pollen.

HOFMEISTER (**17**) made a careful study of all stages in the development of the ovule and confirmed the facts then known. He pointed out how wonderfully simultaneous fertilization occurs in all the plants of the same species and how rapidly the proembryo stages are passed through. He was the first to regard the terminal cell of the early embryo as an apical cell. He thought also that the later embryo and seedling grow by means of an apical cell, and even believed he could demonstrate it in the adult stem tip of conifers.

PFITZER (**32**) denied the existence of an apical cell in the stem tip of conifers, but confirmed HOFMEISTER'S work in regard to the existence of an apical cell in the early embryo, although he assigned to it only about 5 segments as a maximum for *Thuja*, and in Pinaceae he stated that the apical cell stage was even shorter. He calls attention in his conclusion to the fact that this may be taken as a case of embryonic recapitulation of the pteridophyte manner of development. He published no figures.

STRASBURGER (**38**) made a very careful study of the embryogeny of 8 or more genera of gymnosperms. In many particulars he corroborated the former accounts. His many excellent figures

are accurate and most of them still useful. He denies the existence of an apical cell in all but the Cupressineae, where he found a definitely organized apical cell in the early embryo. In *Pinus* and other Abietineae he finds this stage omitted or not constantly present, an indication that these are less primitive than the Cupressineae. In the further differentiation of the embryo he goes into greater detail than any previous worker. In *Pinus* and *Picea* the plerome tip of the root is set off about 0.15 mm. from the apex of the cylindrical mass of cells which is now about 0.5 mm. long, measured to the point where the cells form suspensors. In the account, which he says is practically the same for all the conifers, the stem tip meristem is next in appearance, followed by the cotyledonary primordia which arise in a circle about this point. His description of the cotyledon and stem tip development is substantially the same as that of SCHLEIDEN (**36**). At this stage of development the embryo reaches the lower end of the endosperm, and further growth and elongation bring the radical end of the embryo back to the place of origin of the suspensor.

STRASBURGER states that the number of embryos beginning development may be as high as 20, all but one of which abort in various early stages of development. In *Picea vulgaris* he finds that the 4 rows of cells of the proembryo do not separate, but all 4 of the embryonal cells at the tip of the suspensor contribute to the formation of 1 embryo.

The accounts, by the early workers, of the proembryo stages differ widely. SCHACHT (**35**) shows correctly the completed proembryo when it consists of 4 tiers of 4 cells each with the upper tier open to the egg. STRASBURGER (**38, 39**) attempted to explain the stages between fertilization and this completed proembryo, but, like other early workers, he failed to recognize the nature and extent of the free nuclear divisions. CHAMBERLAIN (**5**) described some details in the development of the proembryo, and later COULTER and CHAMBERLAIN (**9**) figured a more complete series of these stages. FERGUSON (**13**) added still more, working on 6 genera of *Pinus*, and found, as did MIYAKE (**28**) in *Picea*, that the upper tier of 4 cells, in the 8-celled proembryo, divide before the lower. Later, KILDAHL (**19**) found both orders of division in

Pinus Laricio, between the upper and lower tier of this stage, and also made a detailed study of the order and manner of development of walls in the proembryo, a thing which had confused many previous investigators.

COULTER (**8**) and COULTER and CHAMBERLAIN (**9**) described some of the early stages in the developing embryo, and, like STRASBURGER, denied the existence of a true apical cell stage. They also stated that the lower tier of the proembryo may develop into a single embryo, or that the vertical rows of cells frequently become separated to form 4 embryos. One of these may even divide by a vertical wall and the 2 daughter embryonal cells become organically separated (**8**), developing subsequently as 2 separate embryos on the end of the same suspensor. This would give us a very fluctuating program of possibilities in the development of the early embryo of *Pinus*.

SAXTON (**33**), in a study of the embryo of *Pinus pinaster*, gives some of the stages in the development of the embryo between the proembryo and the ripe seed. He concludes that an apical cell stage exists, which develops several segments, and in one case shows an embryo which he estimates as one of 30 cells, which still has an apical cell. He describes as anomalous some of the ordinary stages, and his account is rather incomplete, in many respects less adequate than that of STRASBURGER (**38**), to which he does not refer. SAXTON also finds that "the cotyledon primordia are exactly equal and equivalent in their origin."

Investigation

MATERIAL AND METHOD

The cones of *Pinus Banksiana* were collected from the dunes near Miller, Indiana, during the summers of 1914 and 1916, at weekly intervals during the latter part of June, July, and August. Cones of *P. Laricio* were secured from the parks in Chicago in 1914 and 1916, and from Richmond, Indiana, in 1915. *P. sylvestris* was also secured with the Richmond collections, and *P. echinata* was collected at Conway, Arkansas, during the summers of 1914 and 1915.

The embryos were removed from the ovules in the living condition by dissection under water, and these embryos with their suspensor systems were stained and mounted as permanent preparations. Studies were also made from serial sections cut in paraffin, but most of the drawings accompanying this paper were made from the dissected preparations mounted in Venetian turpentine. The latter were found to be superior to anything else for a study of the coiled suspensors and the further development of the rosette.

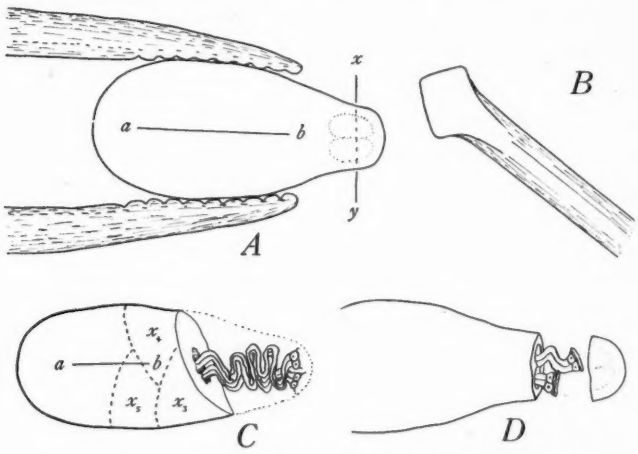

FIG. 1.—Illustrating methods of holding and dissecting pine ovules

DISSECTION.—The dissection must be done with living material under a dissection microscope, or preferably under a binocular microscope with magnification of about 20. The gametophytes are removed from the testa and placed in water in a watchglass. A very useful tool for the dissection, which must be executed under water, is a needle whose point has been flattened and ground to form 2 cutting edges, as shown in *B*, text fig. 1. The naked gametophytes, after being removed from the ovule, are held with forceps in the position shown in text fig. 1, *A*. Frequently the nucellus may be found, resembling a thin cap over the end of the gametophyte, and must first be removed, and sometimes the gametophyte may still be surrounded by the thin inner testa. The

forceps with which the gametophytes are held should be small and have weak springs, in order to avoid crushing the tender tissue. With the dissecting tool *B* the end of the gametophyte is removed along the line *xy*. By teasing a little deeper into the tissue around the edges of the archegonia it is possible to loosen the embryos at the bases of the archegonia, allowing the rosettes to be pushed out by the suspensor as in *D*, text fig. 1.

Usually a little gentle stroking with slight pressure in the direction *a* to *b* with the dissecting instrument held nearly horizontal (to avoid crushing the tissue) is sufficient to loosen the embryo and gradually force it out. A slight pressure with the forceps on the sides of the gametophyte at the proper moment may help. Sometimes gametophytes must be split vertically along the line *ab* before the older embryos can be removed.

When the embryos are imbedded more firmly, it may be impossible to dislodge them by these methods. Sometimes it has been found possible to remove embryos with the complete suspensor system by chipping away pieces of the gametophyte, first from one side and then from the other. This is accomplished most easily by rolling the gametophyte over after each chip has been removed, cutting off pieces x_3 x_4 x_5 (text fig. 1, *C*) alternately, until the embryos are sufficiently loosened. Any method of pulling the embryos out by taking hold of the upper part of the suspensors without first loosening the embryos below results in an incomplete embryo and suspensor system. In spite of the greatest care and perseverance it is often impossible to remove the suspensors and embryos without some of the latter breaking off. Which of the preceding methods is to be used will depend somewhat upon the condition and stage of development of the embryos.

In the earlier studies, which were carried out in this manner, many embryos were found abnormal, in which the protoplasts had escaped from the cells and could be found as dark staining masses near the empty cells. Careful study revealed the fact that this was an osmotic phenomenon, due to the fact that the dissection was executed under water. The cells have a high osmotic pressure, and when placed in water they swell and break in a short time. This may be avoided by dissecting the embryos out under

a 0.3 gm. molecular sugar solution. This strength of solution is still low enough to allow the cells to become fully turgid, and was found satisfactory for a number of species. Doubtless the strength of solution required will vary somewhat with the species and with the condition of the material.

KILLING AND STAINING.—After removal the embryos may be transferred to the killing fluid by means of a pipette with a 2 mm. opening. A good fixing agent is 6 per cent formalin in 50 per cent alcohol, and it is at the same time an excellent preservative in which they may be kept indefinitely, but aqueous formalin alone is not satisfactory. The embryos should be washed through several changes of water before staining, and may be transferred directly to water from the solution. The staining was done in saltcellar watchglasses with Delafield's haematoxylin or safranin. The haematoxylin was used for most of the preparations and was diluted to one-half of its usual strength. The water is removed with a pipette and a few drops of the stain applied for 5–10 minutes, which will stain them very deeply. At this point one of the most difficult steps is encountered, namely, to prevent losing the material while the stain is being removed. It was found best to dilute the stain with water until the watchglass is full. The upper layers of the solution may now be removed without disturbing the embryos at the bottom, but great care must be exercised to prevent losing the embryos, and the material should be watched as the pipette is filled by holding it over an illuminated white surface, as on the stage of a block dissecting microscope. More water is added and the operation repeated until the liquid is clear.

The overstained embryos are now de-stained with acidulated water (about one drop of HCl per 200 cc. of water). The stain is extracted slowly and must be watched over a low power microscope. The de-staining should be continued until the cytoplasm is well differentiated from the nucleus in the embryonal cells at the tip, and the suspensor cells should still be slightly blue. Very thorough washing is necessary to remove all traces of the acid or the preparations will fade. If safranin is used, it is advisable to overstain and then extract the stain to the desired point.

MOUNTING.—After the last washing 10 per cent glycerine is added and the material set aside to evaporate in a place protected from dust. When the concentrated glycerine is washed out with 95 per cent alcohol, great care must be exercised to prevent injury to the preparation. Several changes of alcohol will be necessary to remove all the glycerine, and after washing twice in absolute alcohol the 10 per cent Venetian turpentine is added and the watchglass placed in a desiccator. It is not desirable to allow the Venetian turpentine to get too stiff, as the specimens will be broken in mounting. If more of the 10 per cent Venetian turpentine is added to thin it down, as is frequently done, it causes the cells to swell, the cell walls separating from the protoplasts, leaving a permanent clear space between. If the Venetian turpentine must be thinned down, it should be done by adding about 85 per cent Venetian turpentine. The preparations may be picked up for mounting by means of a needle with a curved point, or a spear point. In handling them they should be picked up in a drop of Venetian turpentine and not by attempting to pull them out.

Preparations were also made by changing the embryos from concentrated glycerine into glycerine jelly. These mounts were not very satisfactory and compare very unfavorably with those prepared in Venetian turpentine.

METHODS FOR SERIAL SECTIONS.—The ovules were prepared for the fixing agent by removing the testa completely from the gametophytes. This can be done without crushing the latter by slicing away one side of the ovule down to the gametophyte with a sharp scalpel, then slicing away the edge, whereupon the gametophyte may be pried out without injury by inserting the point of the scalpel under it. For the early proembryo stages it is not necessary to remove the gametophytes from the testa, but a slice should be cut from one side, or better from opposite sides, to permit good fixation. The older testa cuts with difficulty, and it was not possible to get good sections of *P. Banksiana* when the coat had been left on in stages after the early elongating suspensor.

The naked gametophytes were removed and placed for 20–30 hours in the killing fluid, consisting of a chromic-acetic mixture ($\frac{2}{3}$ per cent chromic, 1 per cent acetic). After washing overnight

in running water they were dehydrated through a close series of graded alcohols, as follows: 5, 12, 20, 35, 50, 70, 85, 95, and 100 per cent. The xylols were also graded, but less closely: 15, 30, 50, 70, 85, and 100 per cent. The material was infiltrated with paraffin by adding the latter a little at a time, and preventing actual contact of the paraffin with the material by means of a perforated cardboard shelf fitted into the vial, a centimeter above the material.

Longitudinal and cross sections were cut serially 10 μ thick and stained by the usual methods employed for iron-alum haematoxylin. A counterstain of gold orange was found very effective in bringing out the otherwise transparent walls. The gold orange is dissolved in the clove oil to saturation. This is then decanted off and about one-fourth the volume of fresh clove oil added. This solution is poured on the slide after it has been stained and cleared in xylol. Only about a minute is necessary to stain the walls; if continued longer it colors the cytoplasm also. The gold orange has a great tendency to crystallize out as the oil evaporates, especially if the stain is too highly saturated. It is therefore advisable to rinse the slide with clove oil, followed by xylol.

In more recent work it was found that very brilliant preparations may be stained with safranin and light green as follows: the safranin must be a concentrated solution in 50 per cent alcohol (a full strength stock solution was used), with the sections left in it 1–3 days. After a rapid washing in 50, in 80, and then in 95 per cent alcohol the sections were placed in light green (about 1 per cent in 95 per cent alcohol) 2–5 minutes. The time for the action of the light green varies with the age of the material, the strength of the stain, and the length of time the sections were stained in safranin. It is desirable, therefore, to stain all the sections of one collection at the same time, and not to mix several collections in one staining. One or two trials will enable one to determine how long to leave the sections in light green, and the remaining slides may be carried through by this time schedule. If left in light green too long, the safranin will be washed out of the nuclei, and if taken out too soon the light green is not impregnated in the cell walls sufficiently to give the desired brilliant contrast. From light

green the slides must be transferred rapidly through 95 per cent alcohol, absolute alcohol, alcohol-xylol, into xylol. The de-staining process is not checked until the sections have reached the pure xylol solution; thus only a short dip should be given into each solution. Although safranin with gentian violet, Delafield's haematoxylin, and iron alum haematoxylin with light green were all tested, they were found much less satisfactory than the iron-alum haematoxylin with gold orange or the safranin with light green.

FORMATION OF CORROSION CAVITY WITHIN GAMETOPHYTE

The first change that is noticeable in the tissue below the archegonia is a starch deposit, which appears in the cells of this region about the time of fertilization, or a few days later. In the living gametophyte this deposit makes the tissue appear opaque, and it gradually spreads down into the central part of the gametophyte until this white opaque region comes to occupy a funnel-shaped region extending downward from the archegonia. About the time the embryos break through the bases of the archegonia the cells at the center of this opaque region break down, at first in the large part of the funnel nearest the archegonia. This forms the beginning of the corrosion cavity, an opening which, as it enlarges, assumes the shape of a slightly flattened trumpet. At the same time the starch-containing zone enlarges and becomes more conspicuous.

Sections like that shown in fig. 1 indicate clearly that the digestive action of an enzyme on the endosperm doubtless precedes the elongation of the suspensor. The embryo is soon pushed so far into this cavity by the elongation of the latter that further elongation can only bring about its well known coiled and twisted condition. The importance of this mechanical action of the suspensor in keeping the embryo pressed into the bottom of the corrosion cavity is better realized when one tries to dislodge some of these embryos by dissection.

Many ovules were examined in which the gametophytes had well developed corrosion cavities, yet no traces of embryos could be found in them, indicating that the archegonia may secrete the digestive enzymes to form the cavity even though the eggs have not been fertilized. Many sections of this kind may be found in

the collections of ovules made from one to two weeks after fertilization. These soon dry up and wither away within the hardening testa, so that one would not include them in the later collections of material if the testa is first removed.

The subsequent enlargement of the corrosion cavity to accommodate the growing embryo is unquestionably due to digestive enzymes secreted by the embryo itself. The archegonia disappear as recognizable structures soon after the primary suspensor has fully elongated. The rosettes are usually found pressed against the top of the cavity, which now includes the space occupied by the archegonia after the latter have broken down. An unfertilized archegonium withers away soon after the formation of the corrosion cavity, its place being marked by a shrunken chip of hardened protoplasm which is often molded into the shape of the lower portion and side of this organ. Later this disappears also.

EMBRYO DEVELOPMENT

This investigation takes up the development of the embryo beginning with the 16-celled stage, which has generally been recognized as the end stage of the proembryo. It is necessary, however, to consider some of the well known earlier stages, and for these facts we will depend upon the results of previous workers which have been reviewed in the historical discussion.

Of the 4 tiers of 4 cells each, the lowest constitutes the embryonal group, each of which is an apical cell of one cutting face; the next tier above constitutes the suspensor group, each of which elongates to form a primary suspensor cell; the third tier has been called the rosette, and its further development has never before been followed out; and the uppermost tier of cells, which have incomplete walls and are in open communication with the egg, sooner or later disintegrate. Fig. 1 shows a longitudinal section through the base of an archegonium after the suspensor cells have begun to elongate and before any of the cells of the embryonal tier have undergone further division. In fig. 38 the embryonal tier has given rise to a tier of cells (e_1) between it and the suspensor, and at the left in fig. 37 an embryonal cell may be seen in anaphase of division.

SUSPENSOR.—The tier of suspensor cells elongates and pushes the tier of embryonal cells into the cavity below. When the suspensor cells have elongated slightly more than in fig. 1, the embryonal cells give rise to the first embryonal tube initials (e_1), and by the time the suspensor cells have elongated to the stage shown in figs. 39 and 40 another transverse wall has appeared in the apical cell below, giving rise to e_2, the second embryonal tube initials. This is soon followed by the elongation of the first embryonal tube initials (fig. 6, e_1) to form tubes like the suspensor cells, the first embryonal tubes. This added part of the suspensor is the secondary suspensor.

Separation of the vertical rows of cells soon follows the division of the embryonal cells, although it may occur earlier, as is the case in fig. 37 at the left. In none of the species of pines studied was a single case found in which the 4 vertical rows of cells did not separate to form 4 embryos. It will be seen from a study of figs. 39, 40, 41, and 44 that the elongating first embryonal tubes are no longer in an even tier, and one of the embryos has already gained the lead in penetrating the endosperm. The struggle for supremacy between the 4 primary embryos of an archegonium is well shown in figs. 40, 41, and 44, while in figs. 43 and 45 two archegonia are concerned.

Since the primary embryos have now separated, we shall regard one of these 4 as the unit for discussion. One of the 4 suspensor cells and all of the cells formed below it by the embryonal cell constitute one primary embryo, while all the embryos produced by an egg will be spoken of as an embryo system.

It is evident from a study of the development of the early part of the suspensor that the primary suspensor tubes never divide to form other tubes or cells. Likewise, an embryonal tube never undergoes division after it has begun to elongate, but an embryonal tube initial cell may divide by a vertical wall before elongation, as e_2 in figs. 6, 8, 14, and 20, or e_3 in figs. 10 and 16. When the embryonal tube initial divides and gives rise to 2 or more cells in a tier, these elongate together into a collateral group of embryonal tubes (figs. 47–50), forming a suspensor division. These suspensor divisions are all parts of the secondary suspensor, but when they

consist of 4 tubes, as in fig. 50, they look very much like the lower part of a group of primary suspensors. For example, if the e_3 group of fig. 50 were studied from sections only, with the upper part of the suspensors confused as they are in fig. 45 (making it impossible to trace any of the tubes back to the rosette), it would be natural to mistake this perfect suspensor division as the group of primary suspensors. It is quite possible that a study of such sections has given rise to the statement that in *Pinus* all 4 of the embryonal cells may contribute to the formation of 1 embryo, or they may form 4 embryos.

The initial cell for the second embryonal tubes (e_2) and for the third and subsequent embryonal tubes are cut off as segments of the apical cell, first by transverse walls, and later as oblique segments. The initial cell of an early embryonal tube may elongate into a 1-celled suspensor division, resembling a primary suspensor cell, or it may first divide by a vertical wall as e_2 in figs. 6 and 8. Fig. 16 shows e_2 as a single elongated cell and e_3 with 3 cells, while fig. 20 shows e_2 of 4 cells. There is considerable variation in the number of cells found in the embryonal tube groups of corresponding suspensor divisions, and variations are frequently found among the individuals of the same embryo system.

After the initial cells of the embryonal tubes begin to divide by vertical walls and elongate to form the suspensor divisions, each succeeding bundle of embryonal tubes consists of more cells than the tier above it (figs. 46–52). Only one exception to this has been found among the 500 or more dissected preparations of various pines, and this one was *P. Laricio*, shown in fig. 24. Here s and e_1 (not shown) are single-celled, e_2 is of 2 cells, and e_3 again 1-celled, while e_4 and e_5 will undergo other divisions before beginning to elongate. Careful examination of many preparations indicates that the separation of the 4 primary embryos precedes the division of any of the embryonal tube initial cells by vertical walls.

The primary suspensor, that is, the first suspensor division, is often collapsed and withered by the time 4 or more divisions have formed. The upper parts of collapsed suspensors are shown in figs. 65 and 68, while fig. 46 still has a turgid primary suspensor. The primary suspensors frequently collapse in about the stage shown

in fig. 46 or soon after, and the cells of the older portion of the secondary suspensor also collapse in turn, so that in an older embryo, like that of fig. 51, the upper part of the suspensor cannot be studied.

In order to determine the amount of variation in the early suspensor divisions, several hundred preparations of *P. Banksiana* were examined and the types of suspensor development noted.

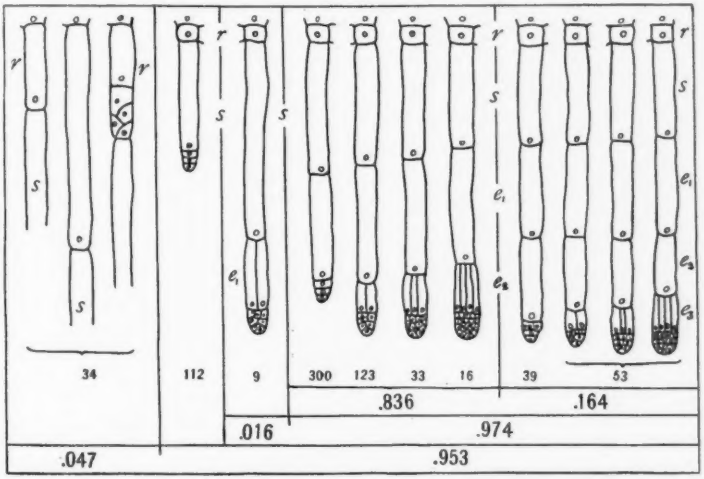

FIG. 2.—Graphic statistical summary of variations in early suspensor divisions of *Pinus Banksiana:* the figures indicate the number of examples of the various types of suspensors observed, and their distribution in percentage.

The results are summarized in the diagrams of text fig. 2. It was common in more than four-fifths of the cases examined to find the single primary suspensor followed by a 1-celled embryonal tube (e_1), this followed by 2 or more cells in the next suspensor division, after which the tubes interlock and elongate irregularly, as in figs. 47, 49, and 51. Less than one-fifth of the cases were found with the primary suspensor followed by 2 successive single-celled suspensor divisions and 2 or more tubes in the fourth suspensor division e_3. Only about 1.6 per cent of the embryos were found to have the first embryonal tubes or second suspensor division of 2 cells.

In nearly 5 per cent of the cases the rosettes were elongated to resemble suspensor cells.

It will be seen that the third embryonal tube group, or fourth suspensor division, always consists of 2 or more cells, and after this division or the one following the tubes begin to elongate and interlock to form the suspensor. The transition from the jointed to the interlocked and more massive portion of the suspensor is well illustrated by figs. 47, 49, 50, and 51.

The suspensor becomes more and more massive as the embryo increases in diameter. The embryo is first pushed as far as possible into the corrosion cavity by the mechanical action of the suspensor; later it remains nearly stationary in the lower end of this cavity, but continues to give off the suspensor by the successive elongation of the cells from the radical end of the embryo; finally, as the embryo develops to its full size, the radical portion again reaches the archegonial end of the cavity. As the root cap becomes differentiated in the embryo, it may be seen that this organ and the suspensor gradually merge into each other; in fact, the late suspensor is formed from the root cap by the successive elongation of layer after layer of cells.

ELONGATED CELLS.—The nuclei of the suspensor cells and embryonal tubes always seem to hold a definite size relation to the cells. A large suspensor tube may frequently contain a nucleus larger than an entire cell in the embryonal group at the apex. The position of these nuclei is always at the embryonal end of these tubes. More of the cytoplasm of the cell is usually found here, near the nuclei. The ends of these cells containing the nuclei are frequently enlarged considerably. Often one of the primary suspensor cells breaks loose at the lower end during elongation. Figs. 41 and 45 show such tubes which continued to enlarge at the lower end and formed a balloon, while fig. 42 shows an earlier stage in another tube. These phenomena are not uncommon.

BASAL PLATE.—A thickened plate (*p*) is deposited above the rosette soon after the suspensor begins to elongate. Something similar was found in *Podocarpus*, where COKER (**6**) calls it a cellulose plug, "a novelty among gymnosperms." It is called a "basal plate" by the writer because it is a plate rather than a plug, and its

chemical nature in *Pinus* was not determined. The word "basal" seems fitting because it is, in a real sense, basal to the embryos in its position. Doubtless careful search will reveal this in many other gymnosperms. Whether the rosette is present as in fig. 41, elongated as in fig. 54, or absent as in *Podocarpus*, the basal plate is always formed in the egg cavity on the walls toward the embryos.

APICAL CELL.—A distinct apical cell stage exists from the time the embryo cells first have walls. In fig. 1 the suspensor cells (*s*) are the first segments of their respective apical cells (*a*). Here the 4 primary embryos are apparently still united; but if they may be looked upon as organizing distinct from each other, the 4 cells which gave rise to the lower 8 cells of the 16-celled embryo are embryo initial cells. The work of COULTER and CHAMBERLAIN (**9**), FERGUSON (**13**), and KILDAHL (**19**) has shown that these which we call embryo initial cells were formed in the mitosis between the 4-nucleate and 8-nucleate proembryo, the place where KILDAHL (**19**) found that the first walls appeared. FERGUSON (**13**) and KILDAHL (**19**) found that the rosette and upper open tier organize next, from the upper 4 nuclei (although KILDAHL found exceptions to this), and therefore this lowest tier of the 12-celled stage is a hold-over since the first appearance of walls.

The second segment of the apical cell is the initial cell of the first embryonal tube. This segment, as well as the third and fourth, are formed by an apical cell of 1 cutting face. Figs. 1–6 all show apical cells of a single cutting face, while in figs. 7, 8, 9, 11, and 12 the first oblique wall of the apical cell has appeared. This wall is sometimes only slightly tilted, as in fig. 9, or it may be nearly vertical, as in figs. 10 and 14.

The stage at which this oblique wall first appears is not always the same. A large number of embryos of *P. Banksiana* were examined in order to determine the average condition in this respect. This study showed that these variations are somewhat similar to those found in the number of tubes in the early suspensor divisions. In nearly two-thirds of the cases the first oblique wall appeared after the primary suspensor and 2 embryonal tube initial cells (3 suspensor divisions) had been formed by the apical cell of one cutting

face; one-fourth after 2 suspensor divisions; and one-tenth after 4 suspensor divisions had been formed.

It is often difficult to determine with certainty in an embryo like fig. 15, for example, at what stage the first oblique wall was formed. Here the last horizontal wall is tilted slightly, so one might think that this was modified by growth after the first oblique wall appeared; but it is also possible that this segment was first formed with a perfectly horizontal wall, and this later enlarged on one side to appear slightly oblique, so that the first real oblique wall is the one which appears nearly vertical. While these two interpretations could be given to fig. 15, in making the study referred to in the foregoing paragraph, the slightly oblique wall was looked upon as though it has been formed in an oblique position by the apical cell.

A stage in which the apical cell has 2 cutting faces does not exist, or it is so shortened that it cannot easily be recognized. Figs. 15 and 16 have only 2 oblique segments cut off, but these are probably the first 2 segments of the apical cell stage with 3 cutting faces. Apical cells with 3 cutting faces are found in embryos only slightly larger, such as figs. 17 and 18. Figs. 17–23 are all from whole mounts in Venetian turpentine and show pyramidal apical cells of 3 cutting faces.

Many irregularities are found in regard to the position of the apical cell. It is frequently so far to one side of the tip of the embryo that it might be overlooked in some serial sections. A section of an embryo like figs. 17, 20, or 28, if cut in another plane, would not show the apical cell so favorably, and might be mistaken for an embryo without an apical cell.

A very puzzling case is shown in fig. 21*a*, *b*. Fig. 21*a* shows the embryo in a high focus, with the shadows of nuclei of a lower focus shown by the dotted lines. Fig. 21*b* shows the nuclei and cell walls of the same as seen in low focus. This looks like an embryo which has no apical cell, and it is on the basis of very similar figures that STRASBURGER (**38, 39**), and other workers since, have denied the existence of an apical cell as a constant feature. In this particular instance the apical cell is at one corner of the lower tier of 4 cells. It is either the cell to the right in high focus, or the lower

cell to the left. For instance, if it is the upper right cell, then either the cell below it or the one in the same plane of focus beside it is its last segment, while the remaining 2 cells together constitute the next to the last segment. The other cells of the embryo may well have arisen while the apical cell had 1 cutting face.

Fig. 13 shows a case very similar to fig. 21*a*, but somewhat younger. If the apical cell and the last segment shown here should both divide with walls in the plane of the paper, and the next tier of 2 cells above this (e_3) should do the same, it would not differ essentially from fig. 21*a*, *b*. Fig. 14 is in the same stage as fig. 13, but with the e_2 suspensor division elongated.

In longitudinal sections the apical cell and its segmentation may usually be seen (figs. 25–29). Fig. 31 is an embryo of about 200 cells, one of the smallest embryos that could be found without an apical cell, and fig. 30 is a larger embryo of about 275 cells, which apparently still has one. Fig. 32 shows a larger embryo of 750 cells which no longer has an apical cell; and figs. 35*a* and 35*b* show the first 2 sections through the end of an embryo in which the apical cell is replaced by a meristematic group. Figs. 34*a* to 34*d* are consecutive cross-sections through an embryo a little larger than that of fig. 32, in which the apical cell may still be found, probably in an arrested condition, before the meristematic group of cells has become active. Fig. 34*e* is a diagram combining sections 34*a* to 34*c* and showing the relation of the segments to the apical cell.

Figs. 33*a* and 33*b*, sections through the tip of an embryo slightly smaller, show an apical cell and segments as diagrammed in fig. 33*c*. This shows the segments arranged clockwise, while in fig. 34*e* they are counter-clockwise. This difference is easily accounted for, since the serial sections on these 2 slides run in opposite directions through the embryos. In fig. 34 the views of the cross-sections proceed toward the apical cell from the base of the embryo, while in fig. 33 they proceed from the apex inward. The segments thus appear in the same order on the embryo and proceed in the same direction as the thread of a wood screw, beginning at the point which corresponds to the apical cell and passing back along the thread toward the older segments. This is probably the usual

arrangement of the spiral of segments, as no exceptions were found in an examination of several other cases, although no extensive study of this feature was undertaken.

The early apical cell forms a slightly compressed and slightly conical mass of cells. When the apical cell ceases to function, as in fig. 32, the embryo is more uniformly cylindrical, sometimes slightly club-shaped. The apical cell vanishes long before the stem tip, the cotyledons, or any of the body regions are recognizable, and nearly all of the early part of the embryo formed by apical cell growth goes to form the suspensor by the elongation of layer after layer of cells from the basal part of the embryo.

ROSETTE AND ROSETTE EMBRYOS

No investigator seems to have followed the development of the rosette further than through the early stages of elongation of the suspensor. That the open cells of the tier above the rosette disorganize has been stated by various workers. The writer has also been unable to find any traces of these nuclei of the upper open tier after the early stages of suspensor elongation, and doubtless they disintegrate.

The rosette has usually been regarded as a group of cells between the main body of the egg and the suspensor, having no particular function. This view has proved to be erroneous, for the rosette is a group of young embryo initials which will produce embryos. These embryos are bounded by thick walls and are not so free to elongate as the primary embryos below them.

After a little delay, during which the adjoining primary suspensor cells elongate, the rosette cells divide, as shown in one of the rosette cells of fig. 58, also in some of the rosette cells seen in polar view in fig. 59. A wall soon appears in one of the 2 daughter cells, inclined at an angle to the first (fig. 61, and rosette of fig. 46), forming the second segment of the apical cell. The apical cell continues to cut off segments on 2 or more sides, and the later embryo appears to have 3 cutting faces. Fig. 65 is a side view of a group of rosette embryos and shows well the apical cell and its segmentation, and (*s*) the upper portion of the collapsed primary suspensor.

None of the rosette embryos has been found to reach stages much in advance of those shown in figs. 64–68. In some of these the embryonal tubes elongating from the basal portion of the embryo have formed a recognizable suspensor, which often appears freakish, as in fig. 68, modified no doubt by the unfavorable position and the unequal thickness of the walls of the rosette cells.

It will be seen that the orientation of these rosette embryos is variable. In fig. 67 they have begun to elongate in various directions. The direction of the apical portion and the suspensor must be determined by the first few divisions, and figs. 59–64 show that these are likewise quite variable. Before the rosette embryos have developed much beyond the early stages, such as fig. 59, the archegonium breaks down, and these embryos may be found pushed up against the top of the corrosion cavity by the suspensor. Even before the archegonium has completely broken down the rosette is frequently tilted by the twisting suspensor below, and it is quite probable that the orientation of the rosette embryos is related to the position of the rosette when the first divisions occur in these embryo initial cells, a thing that may well account for the lack of uniformity or regularity.

It often happens that some of the rosette cells disorganize early and fail to produce embryos. Rosette cells may be found with no visible nuclei, or with nuclei in various stages of disintegration, while the neighboring rosette cells are producing embryos. While these exceptions occur, it is evident that the normal product of an archegonium is 8 embryos. This makes polyembryony a much more extensive phenomenon than has hitherto been recognized. All of the species of *Pinus* investigated showed this peculiarity, *P. Banksiana*, *P. Laricio*, *P. echinata*, and *P. sylvestris*. Rosette embryos develop less rapidly than the 4 primary embryos, abort in early stages, and it is entirely outside of the range of probability that they may ever contribute the embryo of the seed.

Elongation of the rosette.—Another abnormal phenomenon that was occasionally noted was that of elongated rosette cells resembling the primary suspensors. Fig. 53 shows a rosette in the first stages of elongation; fig. 54 shows another that is well advanced. Elongated rosette cells were found in nearly 5 per cent

of the total number of preparations examined in connection with the study summarized in text fig. 2.

A condition which demonstrates that these rosette cells are potentially embryos, even when they elongate to form suspensors or embryonal tubes, is shown in fig. 54, in which a mitotic figure may be seen in the lower portion of one of these elongated cells. Fig. 55 shows this mitotic figure of fig. 54 enlarged. An ordinary suspensor cell or embryonal tube has never been found to undergo division after elongation. The origin of the cells intermediate between the elongated rosette and the primary suspensor of fig. 56, and of 1 rosette in fig. 57, seemed a puzzle until the case shown in fig. 54 made it apparent that these cells may arise from the rosette tube. They are terminal cells of the rosette embryos that were formed after the rosette cell had begun to elongate. The rosette cell at the left, in fig. 53, has a nucleus in spirem stage, probably preparing for the first mitosis in the formation of an elongated rosette embryo of this kind.

POLYEMBRYONY

In *Pinus* polyembryony is a much more extensive phenomenon than is generally known. Since the rosette produces 4 embryos, and 4 others are always produced by the splitting of the lower primary embryos, 8 embryos may be formed from each fertilized egg. The greatest number of embryos possible is 8 times the number of archegonia, which might reach as high as 48 if all 6 of the archegonia, present in some species, were fertilized. Fertilization must be very nearly simultaneous in all the archegonia, and other conditions very favorable if the maximum number of embryos is to be produced. Fig. 69 shows an embryo complex, which had a delayed start and was stunted from the beginning, a condition which is frequently found where more than 3 archegonia are fertilized, with 1 more or less delayed.

In the various pines studied, 4 is the maximum number of embryo sets that were actually found, each related to one of the 4, 5, or 6 archegonia. Two or 3 archegonia were the usual number fertilized. In *P. Banksiana*, with only 2 or 3 archegonia, as large a number is not possible as in *P. Laricio*. Since the cones of the

material studied were poorly pollinated, as was indicated by the relatively few good ovules and seeds developed per cone, no doubt the maximum possible number of embryos was not to be found in these collections.

The terminal embryo of the group is the successful one in the struggle for supremacy among the embryos. In very exceptional cases the successful embryo has been found to be the second one instead of the terminal. Occasionally an embryo develops with the reversed orientation, and the abortive embryos are frequently found in this reversed position.

Cases were also found where less than 4 primary embryos were produced from an archegonium, where one of the vertical rows of cells was aborted with little or no elongation of its suspensor, or the embryo initial cell itself was aborted. This condition might give the impression that one of the 2 or 3 primary embryos is composed of 2 vertical rows of cells that failed to separate in the normal way, were it not for the fact that when one of the embryos aborts in this way there are less than 4 suspensor tubes or first embryonal tubes.

No embryos have been found to arise from 2 or more vertical rows of cells combined. Such an embryo would have 2 apical cells, and wherever an embryo possesses a single apical cell and looks normal in other respects it is safe to conclude that it has come from one of the 4 embryonal cells. Another simple criterion is that of tracing the suspensor back to the rosette. If an embryo could be found attached to 2 primary suspensor cells, without the possibility that an embryo has been lost in dissection, it would indicate that 2 primary embryos were combined, but in this case the embryo should also have the appearance of being double, and the number of embryos present in the complex should be one less than the usual number. The writer found several cases which he suspected to be double embryos, but when they were more carefully studied they failed to fulfil these conditions.

Twins.—So far as I have been able to find, no embryos arise by a further splitting of one of the 4 primary embryos. Since the terminal cell of the early embryo is an apical cell, an equal splitting could only occur after the formation of a vertical wall, as in figs. 10,

13, 14, and 15, and no cases of "twin embryos" formed by such a vertical splitting could be found. The embryos never even show tendencies to round off at these nearly vertical cleavages, or upon the formation of any wall other than the one which separates the 4 primary embryos. Such "twins" would be easily recognized in dissected preparations, since they would be found attached by their secondary suspensors to a common suspensor, leading back to a single primary suspensor cell.

When the 4 primary embryos are sectioned in stages before they are completely separated, it is possible, in rare cases, that 2 embryos may be so cut as to appear to be at the tip of a single suspensor or embryonal tube. This might look as if the 2 embryos had arisen on the end of the same suspensor by the splitting of a single one, especially if some of the adjoining sections are lost. The writer had the opportunity of examining the original slide from which the drawing of a "twin embryo" had been published (**8**). Upon critical examination it proved to show traces of the wall of a second suspensor cell from which the stain had been washed out, and is more correctly shown in fig. 36. One of the adjoining sections, which happened to be a very thick one, is missing from the slide. It was possibly lost off during the staining, as the accidentally thick sections of a series often are, but the recognition of this second suspensor cell gives each of the 2 embryos in this figure its own suspensor and indicates that these 2 embryos were 2 of the primary group of 4, sectioned in a rather unusual position. The possibility that one of the 4 primary embryos could split to form 2 has been claimed by several investigators, but no other figures showing twin embryos of pines could be found in the literature on this subject.

Another type of twins is that found when 2 of the members of the embryo complex develop to fair size to form the mature seed embryos. Although polyembryony is such an extensive phenomenon in *Pinus*, the writer has never been able to find a mature seed with 2 fully and equally developed embryos; one was always considerably larger than the other, and these were not very common. When these 2 embryos are members of the same embryo system, the twin formation is due to a cleavage phenomenon, and is similar to that of duplicate twins in animals.

THE LATER EMBRYO

In embryos of *P. Banksiana*, the size of fig. 47, the apical cell may usually still be found, but by the time the stage shown in fig. 51 is reached it has disappeared. The cylindrical mass of cells

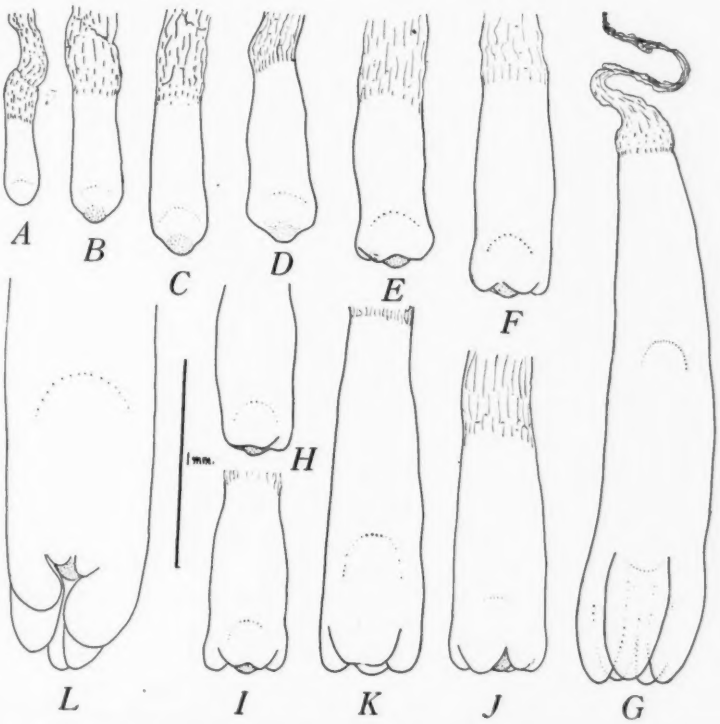

FIG. 3.—Development of stem tip and cotyledons; dotted line represents plerome of root tip; shaded area, meristem of stem tip; *H*, *I*, *J*, *K*, fusing cotyledons.

enlarges, and about the time the stage shown in fig. 52 is reached the cells near the tip begin to organize into an arch, shown by the dotted line of text fig. 3*A*. Under this arch is the plerome of the root tip, the first body region to appear. The periblem organizes outside of this dome and is thickest above it on the side toward the suspensor, where it merges with the tissue of the massive root

cap. This curved cell arrangement may be recognized in the whole embryos mounted in Venetian turpentine or balsam, but sections show the details of this cell organization much better.

The stem tip may be recognized as a slight protuberance in the position formerly occupied by the apical cell, but long after this cell has disappeared. It may first be seen in embryos about 175 $\mu \times 400$ μ; and in living embryos dissected out under water a transparent area develops in the tissues near it, which is shown by the shaded area of *B*, text fig. 3. The embryo enlarges, and by the time it has reached the size of *D* the circle of cotyledonary primordia is recognizable. The number of these primordia, like the number of cotyledons, is not constant, and ranges from 3 to 7. Although the cotyledonary primordia are usually equally developed when they first appear, sometimes they are larger or appear sooner on one side than on the other. Figs. *J* and *K* show cases where 2 primordia formed only 1 cotyledon. Figs. *H* and *I* show the same thing in earlier stages, and since stages older than *K* do not reveal a double tip on the broad cotyledons it is doubtless rapidly outgrown. Many broad cotyledons may have a similar origin, but some of them seem to arise directly from 1 broad primordium. Although embryos like *H*, *I*, *J*, and *K* are not as common as *E* and *F*, those that do not show fusing primordia, there is no doubt a distinct tendency in *P. Banksiana* to reduce the number of cotyledons. The mature embryo frequently has only 3 cotyledons, and 4 or 5 are the usual numbers. In *P. Laricio* fusing primordia were not found, but here there are usually 10 or more cotyledons, and there seems to be no tendency to reduce their number.

The embryos of these 2 species show a tendency to grow slightly zygomorphically. In some cases this seems to date from the first appearance of the primordia. It is usually not very pronounced, but an embryo of *P. Laricio*, extremely abnormal in this respect, is shown in text fig. 3*L*. Here the suppression of the cotyledons on one side is nearly complete, a condition which, in the presence of a cotyledonary tube, would result in an embryo similar to the monocotyledonous embryo, as described in recent work (**11**). Although 2 primordia sometimes combine to form a single cotyledon, none of these pine embryos have a cotyledonary tube at any stage of their development.

ABNORMALITIES

Among the seeds of *P. Banksiana* one was found in a germinated lot which had developed in the reversed position. The cotyledons and hypocotyl were protruding about 15 mm. from the micropylar end of the seed, while the root tip was imbedded in the endosperm. It died without developing much beyond this stage. Some of the aborted embryos of the pine seed are frequently reversed, and LAND (**20**) described a young embryo of *Thuja* which was directed toward the micropyle. Embryos matured in this position are very rare; this case which was germinated was the one case of the kind found in connection with this investigation.

Among the many hundreds of ovules from which the testa was removed preparatory to dissection or imbedding, many cases (at least 15) were found with 2 gametophytes in the same ovule. They occurred in two ways, end to end and side by side. The end to end gametophytes often joined obliquely, and each gametophyte is necessarily formed by the functioning of 2 megapsores. Whether these gametophytes belonged to the same tetrad row or to different tetrads is a matter of conjecture, but one would think that the side by side and obliquely joined prothallia have more probably developed from megaspores of different tetrads. *P. Banksiana*, which was most largely dissected, yielded the most of these double gametophytes. Several were also found of *P. echinata* and two of *P. Laricio*. It is not surprising that a very primitive conifer like *Pinus* should occasionally show this feature.

A few ovules were found in which the terminal embryo aborted and the second one dominated over the others, which is very unusual. Two seeds were found which contained 2 embryos, but in each case the embryo pair was quite unequally developed.

One sectioned ovule of *P. Banksiana* was found in which the customary splitting of the embryo complex did not take place as completely as usual. By a careful study of the series it is clear that each of the 4 embryos is pursuing its own independent development and has its own apical cell. One of the 4 embryos is clearly the largest and will no doubt dominate over the others quite as well as if they were more completely separated.

Discussion

APICAL CELL.—STRASBURGER (**38**) was the first to cast doubt upon the existence of an apical cell in the embryo of the Abietineae. He felt doubtful of it because it did not appear to be a constant feature. The instances in *Pinus* described and figured by him in which he considered the apical cell absent are practically the same as some of the more unusual ones described in this paper. He regards embryos like figs. 13, 14, and 21*a* as having no apical cell; and while he recognized that in embryos like figs. 16 and 18 an apical cell seems to be present, he considered this apical cell growth not constant and that it has no phylogenetic significance.

COULTER (**8**) expresses the opinion that an apical cell is only simulated in *Pinus* and does not in reality exist. He is probably misled by the appearance of nearly vertical oblique walls in the terminal cell and by embryos like figs. 13 and 21*a*. COULTER and CHAMBERLAIN (**9, 10**) do not mention an apical cell, and thus imply that such a stage does not exist, but point out that the problem of the development of the pine embryo after the first few divisions is an open one.

SAXTON (**33**) overlooked STRASBURGER'S work (**38, 39**), and although COULTER had expressed the opinion that an apical cell is only simulated, he is inclined to regard the terminal cell of the *P. pinaster* embryo as an apical cell. When he failed to find an oblique spindle he seemed not fully convinced about the existence of a true apical cell, which he figured only in young embryos up to 30 cells. For these reasons the writer considered it necessary to give considerable study and attention to the proof of the existence of an apical cell in the early embryo.

A series of embryos (figs. 9, 7, 8, 10, 13, and 14) may be selected showing the first oblique wall in all positions, from nearly transverse to vertical. This variation in the first oblique wall has made an occasional embryo hard to explain as having an apical cell. Fig. 15 shows how the next wall comes in, and after this stage the apical cell may easily be found, except that it is frequently very much to one side. The apical cell cuts the first oblique segment at no fixed stage, but probably at the time when the embryo is well separated from its neighbors in the embryo system. This same

cause may also account for the variation in the time of appearance of the vertical walls in the initial cells of the early embryonal tubes.

STRASBURGER (38) also cites the case of an embryo similar to fig. 21*a* as disproving the constant existence of an apical cell, but fig. 21*a* has an apical cell; it is one of the 4 cells of the apical tier, one of the adjacent cells is its last segment, while the two remaining cells constitute the next older segment.

An embryo like fig. 21*a*, but in which the e_2 tier of cells has elongated, looks so much as though it shows the original 4 embryonal cell rows going into a single embryo that doubtless this impression could be created from a study of serial sections only. When STRASBURGER found a stage very similar to fig. 21*a*, however, he was able to trace the suspensor back to a single tube and recognize that it is only one-fourth of the product of the egg. According to his explanation the embryo from this stage on develops like that of *Picea*, in which the whole of the fertilized egg unites to form 1 embryo, and has no apical cell. All of my investigations have failed to support this view, but, on the contrary, embryos slightly older than fig. 21*a*, such as figs. 16, 18, 22, and 28, always have an apical cell, and this cell may usually still be found in embryos of 500 or more cells.[1] Certainly the instances where the apical cell cannot be found in embryos having several hundred cells or less are rare, and the most exceptional cases found in this investigation have been figured and described. Every essential condition for an apical cell is satisfied. It has the proper position on the embryo, being at or near the apex of a body with polar differentiation; it has the same general shape as the apical cell at the stem tip of a fern; and it has recognizable segments which may be related in their regular turn to the 3 cutting faces, even in some embryos of 800 cells.

From a comparative study of the embryos of other conifers it is probable that this apical cell feature is retained more generally than one would suppose. According to STRASBURGER (38) the Cupressineae all have this feature. COKER'S (7) study of the embryo of *Taxodium* does not conflict with this view, for in many

[1] Estimated roughly by counting the average number of cells in diameter and length and applying the formula $l\pi r^2$.

cases he was able to determine the succession of walls in young embryos, which suggests the possibility that an apical cell may be found here. In *Podocarpus* COKER (**6**) figures a number of embryo stages, some of which may have an apical cell; in others it appears doubtful. ARNOLDI (**1**) and LAWSON (**22**) show figures for the early embryos of *Sequoia* and *Sciadopitys* in which the embryonal cell has formed 1 or more vertical walls, a condition which precludes the possibility of an apical cell, according to the views of some investigators. However, according to the later stages of *Sequoia*, figured by SHAW (**37**) and ARNOLDI (**1**), an apical cell arrangement exists, and it is possible that the vertical walls were only the first obliquely placed walls of the apical cell, a condition which occurs occasionally in *Pinus*, and is explained in connection with figs. 13, 14, 15, and 21*a*. SAXTON (**34**) shows some of the stages in *Actinostrobus* which are suggestive of an apical cell, and doubtless it may be found in many of the conifers. It is just as certain to be absent, even in some of the Abietineae, if STRASBURGER'S account of *Picea* (**38**) is correct, for if all 4 cells of the lower tier of the proembryo together produce an embryo, the apical cell loses its identity from the start.

It is evident that in *Pinus* a primitive condition is found, in which the apical cell is still functional for a considerable period, and that in some derived conifers this has been retained more or less, while in some evolutionary lines it has been suppressed or completely eliminated.

APICAL CELL IN RELATION TO PROEMBRYO.—One of the great difficulties in accepting the apical cell as having phylogenetic significance has been the impression that if such a stage may be found it does not exist from the start. In looking over the literature it is apparent that many workers do not recognize an apical cell as such, unless it cuts off oblique segments from several cutting faces. Their apical cell would begin only with the first oblique wall. By studying the behavior of the segments in forming the suspensor the writer has shown that the embryonal cell is a hemispherical apical cell of a single cutting face, and that the primary suspensor cell is its first segment. We need not expect to go farther back in the proembryo than to when the embryo

initial is organized; the stage previous to this is a free nuclear division which organizes these several equivalent cells. Thus the proembryo stage in *Pinus* is the stage in which the divisions occur that bring about cleavage polyembryony.

PROEMBRYO.—The free nuclear division which occurs after the 4 nuclei descend to the bottom of the egg is followed by walls which are complete for the lower tier of cells, but leave the upper tier in open communication with the egg. Thus, when the upper tier divides to form the rosette and the open tier above it, the cleavage is still essentially a free nuclear division. According to FERGUSON (**13**) and KILDAHL (**19**), this upper tier of the 8-celled proembryo usually divides before the lower, and in the resulting 12-celled stage all of the cells with complete walls, namely, the 8 cells of the lower 2 tiers, are embryo initials, which henceforth grow by means of an apical cell. This fact suggests the possibility that the upper open tier may also represent a tier of similar initials which has become abortive. This seems probable when we consider that in *Pinus* the lowest tier produces embryos immediately; the rosette tier only after some delay, and then not always; while the upper open tier represents a group of initials that failed to organize. The presence of this upper abortive tier suggests a reduction from a more extensive form of polyembryony.

The lower tier of the 8-celled proembryo sometimes divides before the upper one, according to KILDAHL (**19**), who also confirms this order. No proof exists that the upper tier ever undergoes another division when the lower one divides first, and it is possible that the nuclei shown in her fig. 11 would have collapsed without undergoing further divisions. This would give us, in this case, only 8 embryo initials instead of 12 (counting the upper open tiers as potential embryo initials), of which only 4 function. It seems evident that this order of division is rather uncommon. According to the interpretation that the embryos are separately organized by means of initial cells in the proembryo, the latter stage has a new significance as a real preliminary stage in the embryogeny. However, the proembryo stage should be considered closed in *Pinus* when the 12-celled stage is reached, rather than the 16-celled stage, and in the instance shown by KILDAHL

the proembryo is complete in the 8-celled stage. Thus far "proembryo" has been recognized largely as a term of convenience to describe the stages preceding the elongation of the suspensor.

POLYEMBRYONY.—The writer has found it necessary to distinguish between the polyembryony caused by the simultaneous fertilization of several eggs and that brought about by the separation of the embryos of a single egg. The latter form of polyembryony, which is spoken of as "cleavage polyembryony," is no doubt a constant feature of *Pinus*, and may possibly be found in some of the other genera of this family. The statement that "all 4 cells of the lower tier may unite to form a single embryo, or they may separate to produce 4 embryos," may hold for the Abietineae as a group. The writer has found separated primary embryos in all of the species of *Pinus* examined, which includes *P. sylvestris*, for which STRASBURGER reports only 1 embryo per archegonium. Forms like *Thuja* (**20**) seem to show splitting of the embryos at times, while in other cases the archegonium produces only 1 embryo. COKER (**7**) found the embryos splitting apart in *Taxodium* and also in *Podocarpus* (**6**). Some of these more modern forms are therefore not constant like *Pinus* in this respect.

Polyembryony by cleavage from 1 egg is no doubt a primitive gymnosperm character, even though it has persisted to the *Ephedra* level, where it is on its way to elimination. No angiosperm has shown this form of polyembryony, which is a further proof that it is a primitive character. Aside from its phylogenetic significance, the feature of polyembryony is a wonderfully effective means for the possible elimination of unfit embryos, involving as it does in *Pinus* some 32 embryos when 4 archegonia are fertilized.

Although no matured twins have been found to arise by the cleavage of the egg in *Pinus*, this has been demonstrated for *Ginkgo* by LYON (**26**). Here we have a close parallel to the animal twins which are formed by cleavage, and LYON has shown that the twin embryos may originate from the same archegonium, remain organically connected, and develop equally to the maturity of the seed.

EARLY EMBRYO IN RELATION TO OTHER CONIFER EMBRYOS.—The known stages of the proembryos in *Picea* (**28**), *Abies* (**29**),

Pseudolarix (30), and *Tsuga* (31) are reported as similar to *Pinus;* the embryo is said to consist eventually of 4 tiers of 4 cells each.

In *Sciadopitys* LAWSON (25) found 8 free nuclei before organization into tiers takes place. This is very significant, for here we may have this extra free nuclear division result in more embryo initials, a thing which would bring about a greater display of cleavage polyembryony than in *Pinus*. Judging from the figures of ARNOLDI (1), this conclusion seems justified, for the central group of cells shown in several of his figures is doubtless made up of many embryo initials from which the embryos are elongating. The writer believes that cleavage polyembryony is a very primitive feature, and it is therefore possible that the embryo of *Sciadopitys* is more primitive than that of *Pinus*.

Little is known in regard to the rosette of other conifers. The work done on *Picea* (28), *Abies* (29), and *Tsuga* (31) does not include the stage showing the suspensor elongating. MIYAKE and YAZIN (30) have figured a stage in *Pseudolarix* with the suspensor elongated, which proves that a rosette group exists in this genus. It is not safe to conclude that a rosette exists in all forms in which the proembryo is organized in tiers like *Pinus*.

In *Pseudotsuga* LAWSON (24) reports a proembryo similar to *Pinus*, but does not show which tier of cells elongates, or whether a rosette exists. He applies the term "rosette" quite generally to the upper tier of free nuclei where no rosette cell group exists. Likewise, COKER, in his work on *Podocarpus* (6) and *Taxodium* (7), uses the term "rosette" to designate the group of free nuclei above the suspensor. While these investigators apply the term "rosette" here, it is evident from a comparison of the figures that a rosette homologous to that of *Pinus* does not exist in *Podocarpus*, *Taxodium*, or *Cryptomeria*. The term "rosette," as first used by MIRBEL and SPACH (27), applies to an unelongated tier of completely walled cells. LAND (20) showed that it is likewise the uppermost tier of completely walled cells that elongates in *Thuja*. The absence of a group of rosette cells and of rosette embryos is a more advanced character, found only in the more recent conifers.

SAXTON (34) has described the embryo of *Actinostrobus*, which repeats the proembryo of *Sequoia* in completely filling the egg

with walled cells. Four of the 6 cells in *Actinostrobus* organize as embryo initials and give rise to embryos. Neither ARNOLDI (1) nor LAWSON (22), in their work on *Sequoia*, followed the embryo development very far. They probably studied the development of only a single embryo from each egg. It seems probable that the other 3 cells which are cut off by walls in the first 2 divisions of the proembryo of *Sequoia* may represent embryo initials, and more careful study may perhaps reveal secondary embryos arising from 1 or more of these other 3 cells. Like the rosette embryos in *Pinus*, these possible secondary embryos in *Sequoia* may develop only after some delay, and thus easily be overlooked. *Actinostrobus*, and possibly *Sequoia*, represent forms in which cleavage polyembryony has been retained more or less.

The cleavage polyembryony of *Pinus* suggests an explanation of the proembryo of *Ephedra*, described by STRASBURGER (38) and LAND (21). Here the 8 free nuclei of the proembryo organize with walls as embryo initials, and from 3 to 5 of them produce embryos. The embryo initials organize only at the bottom of the egg in *Pinus*, while in *Ephedra* they organize with walls before reaching the bottom. *Ephedra* has thus retained, in a modified form, a very ancient character, that of cleavage polyembryony, a character which indicates that this plant has descended from the Coniferales. According to the testimony of their embryogeny, such forms as *Pinus* and *Actinostrobus* must be looked upon as the nearest conifer relatives of *Ephedra*.

A comparative study of conifer embryos suggests several possible evolutionary lines of advance. One of these is the one beginning with *Pinus* and culminating in *Ephedra*, in which cleavage polyembryony is retained in some modified form. The apical cell feature is retained among the more primitive embryos of this line, but apparently lost by the time the *Ephedra* level is reached.

The abietineous embryo of the type represented by *Picea* (38) would be produced when all the embryo initials together develop 1 embryo. Here the lower tier is an even one, and if the embryo develops uniformly a meristematic group of 4 cells replaces the apical cell from the first. Thus *Picea* may represent the culminating abietineous embryo type, while *Pinus* represents the primitive type.

In his work on *Cephalotaxus* STRASBURGER (**38**) shows in pl. 19, fig. 53, what is possibly a rosette embryo group, although he does not refer to it in the text. It is of interest in this connection to note also that the *Cephalotaxus* embryo has a cap which associates it with *Araucaria* (**4, 38**) and *Agathis* (**12**). All of this suggests another possible line of advance from a *Pinus*-like ancestor, through intermediate forms like *Cephalotaxus*, and a culmination in the embryo of the araucarian type. Thus it looks as though nearly all the embryos of Coniferales may be derived from an ancestor with cleavage polyembryony and an apical cell like *Pinus*, differentiating into the several more or less distinct lines of specialization. This is a strong argument in support of the theory that *Pinus* is a very primitive and ancient genus.

POLYCOTYLEDONY.—If polycotyledonous gymnosperms have been derived from dicotyledonous ancestors, one would expect that in the ontogeny of the cotyledons 2 primordial zones would first appear, and these 2 zones divide up and give rise to the primordia of the separate cotyledons. On the other hand, this investigation goes to prove the opposite; namely, that the polycotyledonous condition is the more primitive, and the tricotyledonous or dicotyledonous condition derived.

Most of the work which has been done on polycotyledony has been based upon the vascular anatomy of the seedling (**16**). The arguments that favor the derivation of polycotyledonous embryos by a splitting of cotyledons are based on anatomy and are well summarized by COULTER and CHAMBERLAIN (**10**), who state that "it must be remembered that these same facts may be used also as evidence that the dicotyledonous condition has arisen from the fusion of more numerous cotyledons."

SAXTON (**33**) also doubts the origin of polycotyledons from dicotyledons, and concludes from a study of cross-sections of *P. pinaster* that "the primordia are exactly equal and equivalent in origin." However, he produced no direct evidence to indicate that fusions of the many cotyledons may have occurred.

The study of the ontogeny of the cotyledons brings out facts not hitherto considered in connection with this problem. In speaking of cotyledonary fusions, it must be understood that full

grown cotyledons did not fuse, but 1 cotyledon is developed in the place formerly occupied by 2. The number of cotyledons is actually reduced by fusion of the primordia.

A zygomorphic tendency, which is usually only very slight, is evident in nearly all mature embryos of *P. Banksiana*, but only occasionally in the early stages of the embryo. This zygomorphy of the matured embryo is, no doubt, a secondary result due to the shape of the seed, for it is always oriented within the seed in the same manner, and the zygomorphy is less pronounced in the case of *P. Strobus*, which has a more regularly shaped seed. The zygomorphic tendency found in some early embryos cannot be related to the shape of the seed, and is no doubt due to certain hereditary tendencies. The most extreme case found (text fig. 3*L*) was that of *P. Laricio*. When zygomorphy is pronounced, as in this case, it furnishes an interesting parallel to the development of certain monocotyledonous embryos at the stage when primordia develop, as shown recently by COULTER and LAND (**11**). In *Pinus* the zygomorphy never goes to such an extreme as in the monocotyledonous embryo, and no cotyledonary tube is formed in any of the pines that were studied. That we have well developed cotyledonary tubes among the Abietineae is shown by the work of HILL and DEFRAINE (**16**) and by the recent work of HUTCHINSON (**18**) on *Keteleeria*. The cotyledonary tube would be formed as a natural accompaniment of a coalescence of the many cotyledons by fusion; its very existence among the historically recent gymnosperms is a further indication that cotyledonary fusion has taken place, rather than a splitting. It is interesting to note in this connection that the number of cotyledons in *Keteleeria* is 4, a rather reduced number.

Summary

1. A special technique for dissecting ovules, staining and mounting the embryos, and an improved method of staining embryos in serial sections have been described in detail.

2. The corrosion cavity results from an enzyme, which may be secreted by the unfertilized eggs as well as the embryo.

3. Two forms of polyembryony must be recognized in gymnosperms, namely, cleavage polyembryony and the polyembryony

due to pleurality of archegonia. In *Pinus* one usually finds both types associated in the same ovule, and cleavage polyembryony always occurs in the several species of *Pinus* that were investigated. It is probably a constant feature of this genus.

4. The rosette consists of a group of embryo initials which usually produce embryos. Rosette embryos, like 3 of the 4 primary embryos, are always aborted.

5. Each embryo of a system may be traced back to an initial cell, one of the first completely walled cells of the proembryo. The 8 embryos formed by the cleavage of the egg are therefore definitely organized from the time of the last free nuclear division.

6. A further splitting of one of these 8 embryos into "twins" was not found to occur in *Pinus*. In rare cases 2 matured embryos were found in an ovule, but they were very unequal and due simply to the incomplete dominance of a single embryo.

7. The early embryo develops by means of an apical cell which exists from the time the first walls appear in the proembryo. This apical cell persists for a considerable period, being still recognizable in embryos of 500–700 cells.

8. The apical cell represents a primitive fern character, which is recapitulated in the embryogeny of *Pinus*.

9. Less than 4 primary embryos per archegonium may be produced in case one of the embryo initials, or the early apical cell, disorganizes.

10. The suspensor is formed by the elongation of cells in the basal portion of the embryo, a process that begins with the elongation of the first apical cell segment and continues until the maturity of the embryo.

11. Suspensor cells or embryonal tubes never divide after elongation, but rosette cells may elongate and later divide in forming the rosette embryos, showing their greater potentialities and their distinctness from the suspensor cells which they resemble.

12. Considerable variation occurs in the first secondary suspensor divisions, also in the time of appearance of the first oblique walls formed by the apical cell; both are doubtless related to the time of separation of the embryos.

13. Cleavage polyembryony is a primitive character which *Pinus*, *Sciadopitys*, *Actinostrobus*, and doubtless other genera have

retained. *Ephedra* has also retained it in a modified form, and this definitely associates Gnetales with the Coniferales rather than the cycads.

14. The other evolutionary lines suggested in the discussion likewise assign a primitive position to *Pinus*, so that this ancient type seems to be genetic to several conifer lines.

15. The body regions of the later embryo, so far as they have been determined, appear in the following order: plerome tip of root, periblem and root cap, stem tip, and cotyledons.

16. There is a distinct tendency in *P. Banksiana* toward a reduction in the number of cotyledons, attested by the fact that 2 primordia have been found to form 1 broad cotyledon. This suggests that the dicotyledonous condition has been derived from the polycotyledonous condition through cotyledonary fusions.

17. Cotyledonary tubes are the result of past cotyledonary fusions, and are found in embryos between the primitive polycotyledons and dicotyledons.

18. The zygomorphic feature of a monocotyledonous embryo is foreshadowed in the embryo of *Pinus*.

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DESCRIPTION OF PLATES VI–X

Figures of plate VI from dissected preparations, except figs. 1–5; figures of plate VII all from serial sections; figures of plates VI and VII ×300; figures of plates VIII–X drawn to same scale from dissected preparations and ×80; lettering in all figures as follows: *a*, apical cell; *e*, embryonal tubes; e_1, first embryonal tube (or its initial cell if it marks an unelongated cell); e_2, second

embryonal tubes; e_3, third embryonal tubes; *o*, portion of egg containing upper open tier of nuclei; *r*, rosette cells; *s*, suspensor (primary suspensor); *p*, basal plate (plate of thickening usually formed above rosette); all figures of *Pinus Banksiana* unless otherwise indicated.

FIG. 1.—Section through base of archegonium, showing suspensor cells (*s*) elongating before lower tier of tip cells (*a*) found in 16-celled proembryo have divided; early corrosion cavity forming; June 20, 1914.

FIGS. 2–5.—Sections through 4 separated embryos all coming from the same egg, still even with each other, but with their apical cell mitosis not simultaneous; mitosis shown results in second embryonal tube initial (e_2); June 20, 1914.

FIG. 6.—Vertical wall forming in second embryonal tube initial, which will result in a 2-celled suspensor division, as shown in fig. 8.

FIG. 7.—Later stage than fig. 6, in which an oblique wall has been formed by apical cell and no vertical wall has yet appeared in any embryonal tube initials.

FIG. 8.—Later stage than fig. 6, in which a 2-celled suspensor division has begun to elongate; first oblique wall cut off by apical cell has just been formed; June 30, 1916.

FIG. 9.—Later stage, in which first oblique wall of apical cell is only slightly tilted; 2-celled embryonal tube division has become well elongated; July 1, 1916.

FIG. 10.—Apical cell of *Pinus Laricio*, forming first oblique wall, in this case almost vertical; July 6, 1916.

FIGS. 11, 12.—Usual appearance of embryos with first oblique wall formed by apical cell; June 29, 1916.

FIGS. 13, 14.—Occasional appearance of embryo after vertical wall has been formed by division as shown in fig. 10; in fig. 14 embryonal tube initials (upper cells of group) have elongated, leaving only 4 cells below; fig. 13, June 22, 1914; fig. 14, July 1, 1916.

FIG. 15.—*Pinus Laricio*, showing how second oblique wall is formed by apical cell after first has appeared vertical; July 16, 1916.

FIG. 16.—Usual condition after first 2 oblique segments have been formed; July 1, 1916.

FIG. 17.—Later stage with distinct apical cell placed slightly to one side; apical cell has 3 cutting faces; June 30, 1916.

FIG. 18.—Usual condition of slightly older embryo; June 29, 1916.

FIGS. 19, 20.—Embryos with apical cell in rather unusual position; June 30, 1916.

FIG. 21.—Two views of same embryo; *a*, in a high plane of focus, showing shadows of lower nuclei; *b*, showing only nuclei of lower plane of focus and walls; apical cell difficult to distinguish with certainty; June 22, 1914.

FIGS. 22, 23.—Older stages than last, with distinct apical cells; July 5, 1916.

FIG. 24.—*Pinus Laricio:* unusual suspensor in which third embryonal tube initial (e_3) remained undivided, although suspensor section above it has 2 collateral tubes; very exceptional; July 6, 1916.

FIGS. 25–27.—Longitudinal sections showing successive stages in development of embryo by apical cell with well marked segments; June 30 and July 5, 1914.

FIG. 28.—Embryo about same stage as fig. 26, but with apical cell placed very much to one side; if section had been cut longitudinally and at right angles to this plane, apical cell would have been obscured; July 5, 1916.

FIG. 29.—Very late stage, in which apical cell is still active and segments very distinct; July 5, 1914.

FIG. 30.—Late stage of embryo with apical cell which has probably become inactive and is on verge of being eliminated; July 5, 1914.

FIG. 31.—Embryo smaller than fig. 29, which no longer possesses an apical cell.

FIG. 32.—Embryo past apical cell stage; July 5, 1914.

FIG. 33.—Two successive cross-sections through the tip of an embryo 100 $\mu \times 180\ \mu$, in which an apical cell may still be found; diagram 33*c* shows relations of segments; July 12, 1916.

FIG. 34.—Three successive cross-sections (*a*, *b*, *c*) through tip of an embryo 128 $\mu \times 280\ \mu$ (larger than any other embryo shown on this plate) in which apical cell may still be found, although doubtless it has become inactive; *d*, section through widest part of same embryo; *e*, segmentation as reconstructed from *a*, *b*, *c;* July 8, 1916.

FIG. 35.—Two successive sections through tip of embryo 108 $\mu \times 200\ \mu$, showing no trace of apical cell.

FIG. 36.—*Pinus Laricio:* drawing of an embryo described (**8**) as coming from splitting of a single embryo on end of a single suspensor cell, showing faint wall of another suspensor cell to right; it is no doubt a case where 2 primary embryos have not completely separated and are sectioned in an unusual position.

FIG. 37.—Two embryo groups of neighboring archegonia in early stage of suspensor formation; apical cell of embryo to left is in mitosis giving rise to first embryonal tube initials; all other embryos have already formed these cells; separation of embryos evident; June 29, 1916.

FIG. 38.—Embryos of same age showing early separation of the 4 primary embryos of each archegonium.

FIGS. 39, 40.—Successive stages in elongation of suspensors, first embryonal tubes, and separation of 4 primary embryos of archegonium; June 26–30, 1916.

FIG. 41.—More complete drawing of later stage with completely separated embryos and partly elongated embryonal tubes; one of the 4 primary embryos has broken loose from its attachment below and enlarged into a balloon at

lower end containing the nucleus; thick deposit of material, basal plate (*p*), is shown on upper wall of rosette; June 24, 1916.

FIG. 42.—Suspensor, of which lower end is beginning to enlarge into a balloon; June 24, 1916.

FIG. 43.—Embryo complex from 2 adjacent archegonia with 8 primary embryos present and 1 set of rosette embryos forming; all first embryonal tubes have elongated and some of second embryonal tubes are about to elongate; June 30, 1916.

FIG. 44.—Embryos from 1 archegonium of about the same stage as fig. 43; 1 embryo has been left far behind in the "struggle for supremacy"; June 22, 1914.

FIG. 45.—Embryo complex similar to fig. 43, but rather more advanced; a balloon-like enlargement may be seen at end of 1 primary suspensor; one of the 8 embryos has been aborted and one left far behind; July 1, 1916.

FIG. 46.—Embryo system from 1 archegonium in which primary suspensors and first embryonal tubes of secondary suspensors have fully elongated, while next divisions of suspensors are nearly half elongated; July 1, 1916.

FIG. 47.—Embryo in which third division of suspensor has completely elongated and succeeding portions of suspensor are beginning to form embryonal tubes of unequal lengths that break joints; suspensor slightly crushed below, thus separating embryonal tubes; July 5, 1916.

FIG. 48.—Embryo slightly older than in fig. 47, but with less developed suspensor becoming massive very suddenly; June 29, 1916.

FIG. 49.—Embryo with very typical suspensor forming fourth suspensor division (third secondary portion), with young embryonal tubes beginning at base; July 5, 1916.

FIG. 50.—Embryo of somewhat older stage than fig. 49.

FIG. 51.—Later massive embryo with characteristic secondary suspensor made up of dovetailed embryonal tubes (or tubes that break joints) in which suspensor divisions no longer appear; July 8, 1916.

FIG. 52.—Older embryo than fig. 51 shortly before differentiation of body regions; July 8, 1916.

FIG. 53.—Rosette in early stage of elongation (cases of elongating rosette cells are found in 5 per cent of embryos of *P. Banksiana*).

FIG. 54.—Rosette fully elongated, with a mitotic figure in lower end of one of its cells; June 30, 1916.

FIG. 55.—Detail of lower end of elongated rosette of fig. 54, showing division spindle.

FIG. 56.—Rosette elongated and divided into embryo of many cells, of which figs. 54 and 55 was a delayed beginning; June 30, 1916.

FIG. 57.—Rosette embryo similar to fig. 56, with suspensor tube broken off from below.

FIG. 58.—Embryo system with first division of rosette embryo showing in one of rosette cells, beginning of usual type of development of rosette embryos; June 27, 1916.

FIGS. 59–61.—Views of rosettes from above, showing stages in development of rosette embryos; June 29–30, 1916.

FIG. 62.—Rosette embryo of *Pinus echinata* in oblique view, showing apical cell; July 23, 1914.

FIG. 63.—Views of rosettes of 2 adjacent archegonia as seen from above, showing different stages in which various rosette embryos may be found at the same time; June 30, 1916.

FIG. 64.—Later rosette embryos well developed, but no tubes elongated to form a suspensor; July 8, 1916.

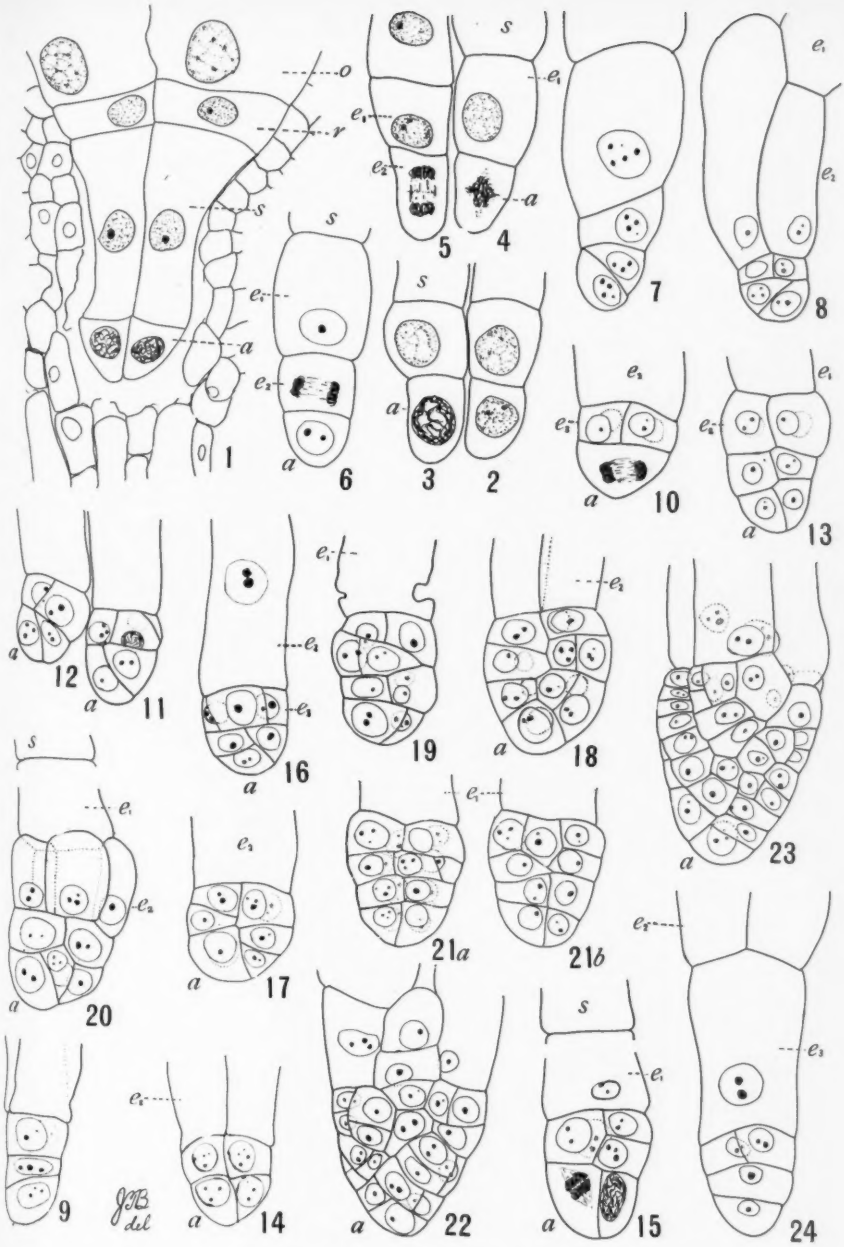
FIG. 65.—Side view of group of rosette embryos, one of which shows an apical cell and distinct segmentation; primary suspensor (*s*) of lower 4 embryos has entirely collapsed by the time this stage is reached; July 8, 1916.

FIG. 66.—Side view of rosette embryos from 2 adjacent archegonia, from some of which embryonal tubes have elongated to form a suspensor, showing that rosette-cell proliferations are real embryos.

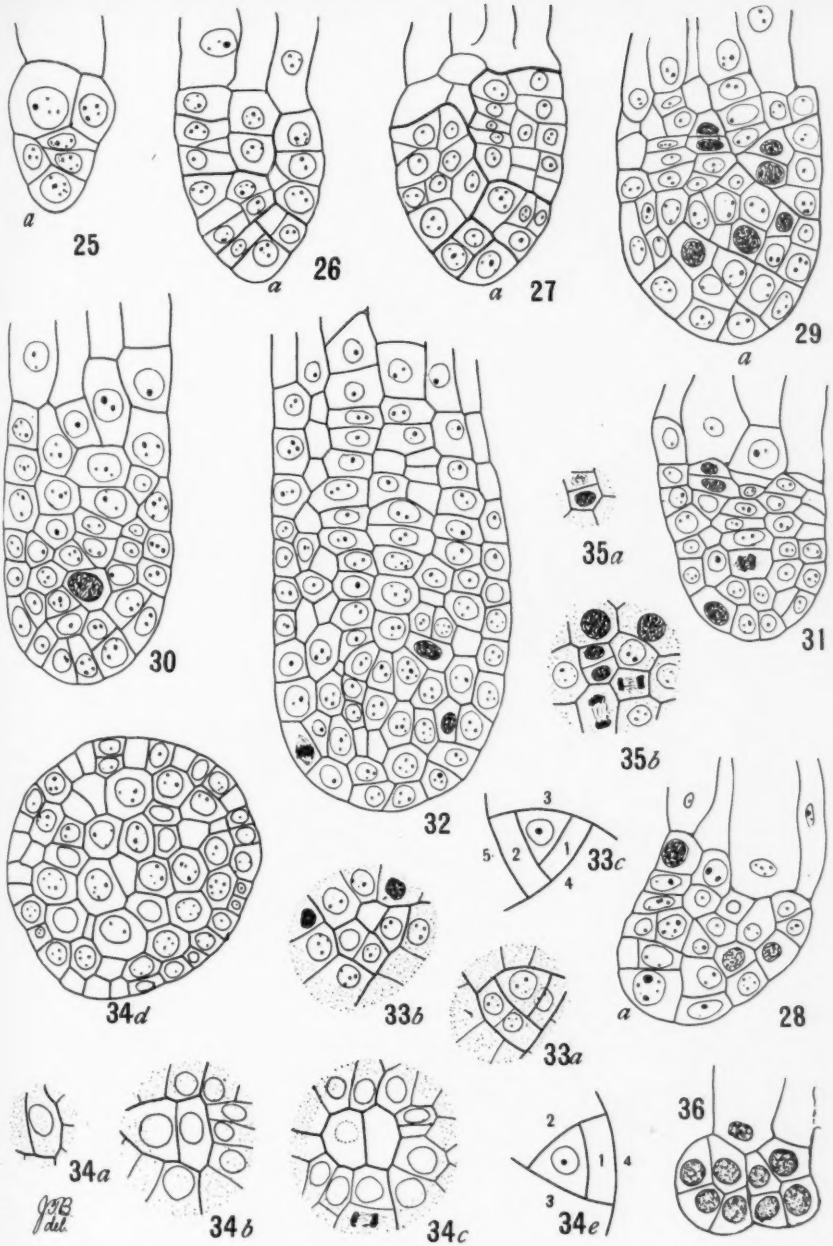
FIG. 67.—Group of rosette embryos which have suspensors elongated in various directions, although number of cells formed is less than in fig. 64, where no elongation has thus far occurred; July 5, 1916.

FIG. 68.—Rosette group showing 1 embryo with suspensor elongating under difficulty and distorted, on account of heavy wall found between rosette and suspensor cells.

FIG. 69.—Embryo system which was badly stunted, due to delay in fertilization or development, while 2 or more adjacent embryo systems gained supremacy; June 29, 1916.



BUCHHOLZ on PINUS



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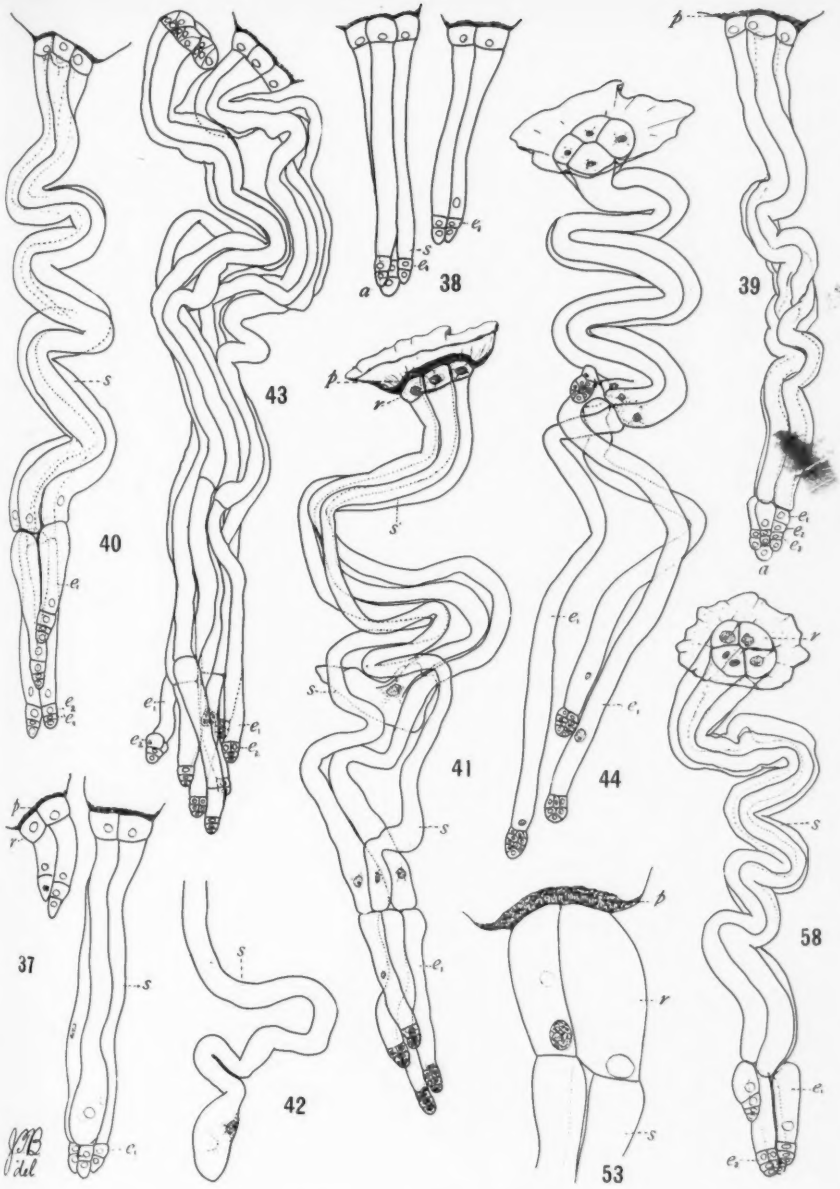

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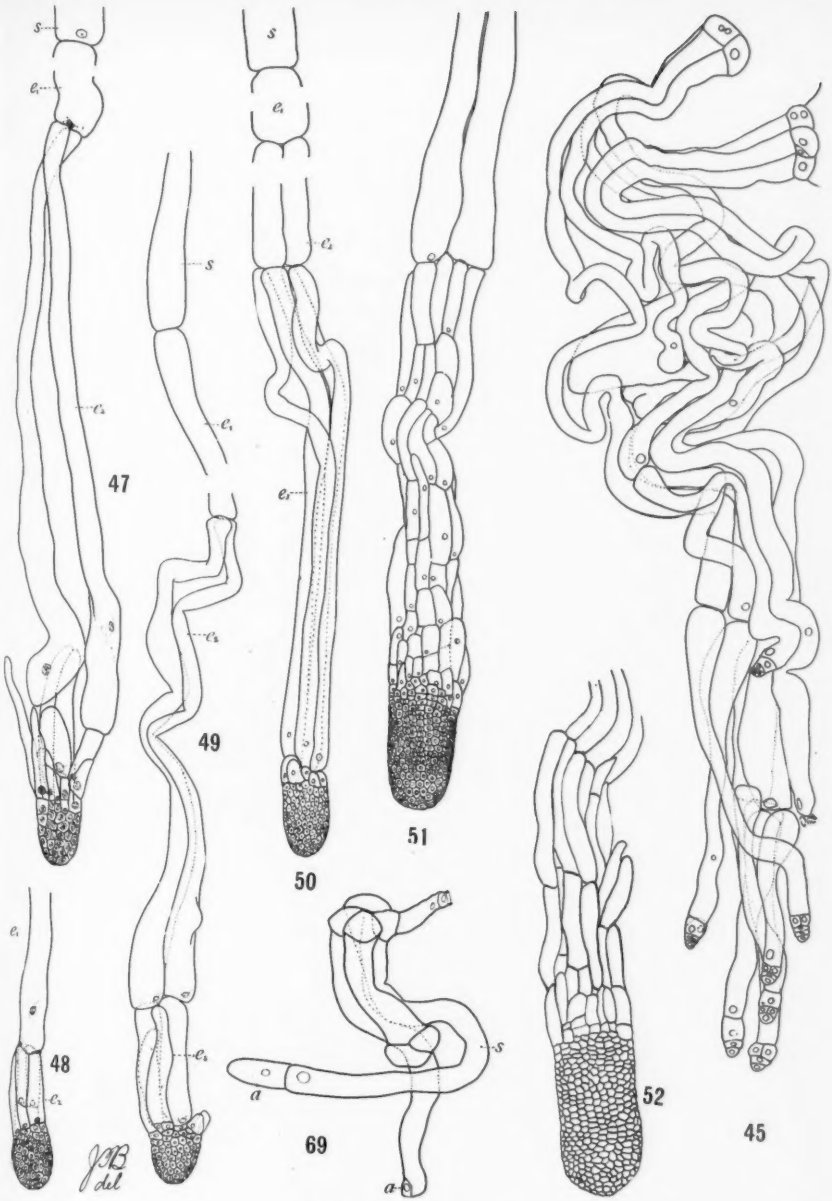

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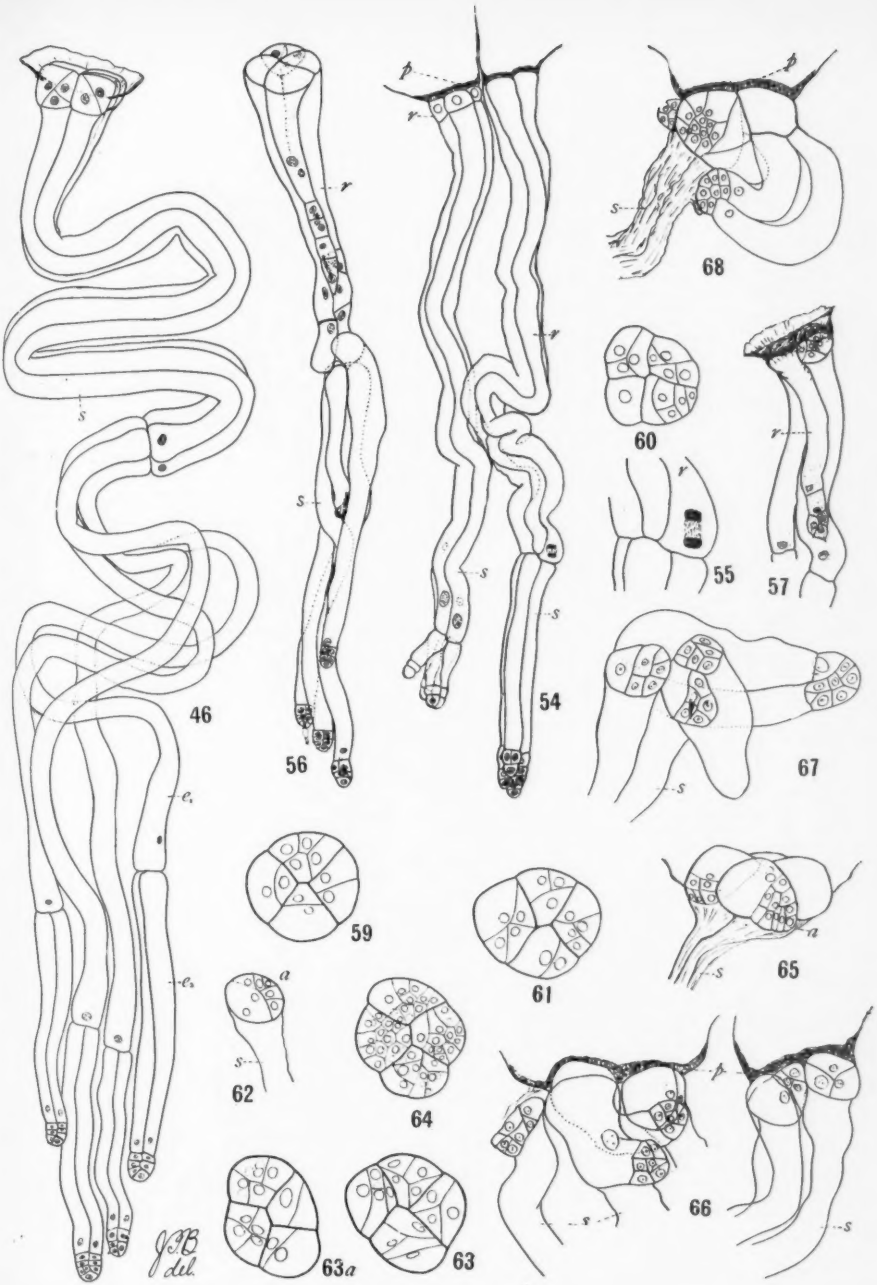

BUCHHOLZ on PINUS

NOTES ON NORTH AMERICAN TREES. II. CARYA

C. S. SARGENT

Conspectus of the species of the United States

Bud-scales valvate, the inner strap-shaped and only occasionally slightly accrescent; fruit more or less broadly winged at the sutures (*Apocarya* C. DC.).

Shell of the nut thin and brittle; leaflets more or less falcate.

Aments of staminate flowers nearly sessile, usually on branches of the previous year; lobes of the seed entire or slightly notched at apex.

Leaflets 13–15; nut ovoid-oblong, cylindrical; seed sweet . . 1. *C. pecan*

Leaflets 7–11; nut oblong, compressed; seed bitter . . . . . 2. *C. texana*

Aments of staminate flowers pedunculate, on branches of the year or of the previous year; lobes of the bitter seed deeply 2-lobed.

Leaflets 7–11; nut cylindrical or slightly compressed . . 3. *C. cordiformis*

Leaflets 7–13; nut compressed, usually conspicuously wrinkled 4. *C. aquatica*

Shell of the ellipsoidal nut thick and hard; lobes of the sweet seed deeply 2-lobed; leaflets 7–9, occasionally 5, rarely slightly falcate; aments of staminate flowers long-pedunculate at the base of branches of the year . . . . . 5. *C. myristicaeformis*

Bud-scales imbricated, the inner becoming much enlarged and often highly colored; aments of staminate flowers on peduncles from the base of branches of the year, rarely from the axils of leaves; fruit usually without wings; seed sweet, its lobes deeply 2-lobed (*Eucarya* C. DC.).

Branchlets usually stout, slender in no. 7; involucre 6–12 mm. in thickness, opening freely to the base.

Bark on old trunks separating into long, broad, loosely attached scales; nuts pale.

Branchlets light red-brown; shell of the nut thin.

Leaflets 5 or rarely 7, obovate to ovate, acute or acuminate; nut much compressed, often long-pointed at apex; branchlets glabrous or pubescent . . . . . 6. *C. ovata*

Leaflets 5, lanceolate, acuminate; nut little compressed, acute at apex; branchlets slender, glabrous . . . 7. *C. carolinae-septentrionalis*

Branchlets pale orange color, pubescent; leaflets usually 7–9; shell of the nut thick . . . . . 8. *C. laciniosa*

Bark not scaly, on old trunks dark, deeply ridged; leaflets 7–9, often subcoriaceous, pubescent below; nut reddish brown, often long-pointed, thick-shelled; branchlets pubescent . . . . . 9. *C. alba*

Branchlets slender; leaves 5–7-foliolate; involucre of the fruit tardily dehiscent to the middle, indehiscent or opening freely to the base; shell of the nut thick, bark close or sometimes scaly in no. 13.

Branchlets and leaves not covered when they first appear with rusty brown pubescence.

Involucre of the fruit 3–5.5 mm. in thickness, opening freely to the base; leaves usually 7-foliolate; winter-buds pubescent.

Leaflets hoary tomentose below in early spring, slightly pubescent at maturity; petioles and rachis glabrous; fruit broad obovoid; branchlets glabrous.....10. *C. leiodermis*

Leaflets covered in early spring with silvery scales, pale and pubescent below during the season; petioles and rachis more or less thickly covered with fascicled hairs; fruit ellipsoidal to obovoid or globose; branchlets glabrous or slightly pubescent.....11. *C. pallida*

Involucre of the fruit 1–3 mm. in thickness; winter-buds glabrous or puberulous.

Leaves 5-, rarely 7-foliolate, glabrous or rarely slightly pubescent; fruit obovoid, often narrowed below into a stipitate base, the involucre indehiscent or tardily dehiscent.....12. *C. glabra*

Leaves generally 7-foliolate, glabrous or rarely pubescent; fruit short-oblong, subglobose or obovoid, the involucre opening freely to the base; bark often more or less scaly..... 13. *C. ovalis*

Branchlets and leaves densely covered when they first appear with rusty brown pubescence; leaflets usually 5–7; winter-buds rusty pubescent.

Fruit obovoid; the involucre 2–3 mm. in thickness; peduncles of the aments of staminate flowers often from the axils of leaves; branchlets soon becoming glabrous.....14. *C. floridana*

Fruit subglobose to broadly obovoid, ellipsoidal or pyriform, the involucre on the different varieties 2–13 mm. in thickness; branchlets pubescent through their first season.....15. *C. Buckleyi*

1. CARYA PECAN Engl. and Graeb.—The pecan was evidently planted by the Indians in the Mississippi Valley, and it is sometimes difficult to determine the natural distribution of this tree. It is probably indigenous in western Mississippi and in West Feliciana Parish, Louisiana, but the statement in my *Silva of North America* that the pecan extended to central Mississippi and Alabama must, I think, be taken to refer to planted or possibly naturalized trees; and it is possible that some of the pecan trees in southern Indiana, especially those toward the Ohio border, were planted by the Indians or are descendants of their trees. Westward in the United States the pecan ranges to the valley of the south fork of the

Arkansas River in Woods County and to Comanche County, Oklahoma, and in Texas to the valley of the Devil's River and to Hardiman County.

2. CARYA TEXANA C. DC.—In addition to the stations in Texas and Arkansas (see Trees and Shrubs **2**:206) the bitter pecan has been found near Lake Charles in Calcasieu Parish, and near Laurel Hill, West Feliciana Parish, Louisiana, and near Natchez, Adams County, Mississippi.

3. CARYA CORDIFORMIS Schn.—This is perhaps the most widely distributed although not the commonest of all the species of *Carya*, as it ranges from southern Maine to the valley of the St. Lawrence River near Montreal and to that of the Ottawa at Hull in the Province of Quebec, and westward to northeastern Nebraska and eastern Oklahoma, and southward to western Florida, northern Alabama, western Mississippi, Louisiana, and eastern Texas. In New England it appears to grow farther north than the other species, but in the valley of the St. Lawrence River in Quebec and Ontario it is associated with *C. ovata*. It grows on the shores of the Straits of Mackinac, Michigan, and in southeastern Minnesota with *C. ovata*, but as it ranges much farther north than that species in Minnesota it must be considered the most northern representative of the genus. In the south Atlantic states from Virginia to southern Georgia it is found from the coast up to altitudes of about 700 m. on the Appalachian Mountains. I have no record of its occurrence in Florida outside the valley of the Apalachicola River or in the coast region of the Gulf states.

To persons who have not read books about trees *C. cordiformis* is generally known, at least in the southern states, as the pignut, and this name should be used for it rather than for *C. glabra* or *C. ovalis* and their varieties, which all have sweet and edible seeds. The first published account of *C. cordiformis* appeared in 1731 in CATESBY'S *Natural History of Carolina* (**1**:38. *pl. 38*), where it is called "*nux Juglans Carolinensis fructu minims putamiene laevi*, the pignut." CATESBY describes the nuts "as not one-quarter part so big as those of the hickory, having both inner and outer shells very thin, so that they may easily be broken with one's fingers. The kernels are sweet, but being small and covered with a very

bitter skin, makes them worthless except for squirrels and other wild creatures." CATESBY's figure of a single nut is not a very good one, and at one time led me to suppose that he had figured a nut of some form of *C. ovalis*, but there can be no doubt that his description is that of *C. cordiformis*. In 1770 MUENCHHAUSEN (Hausv. **5**:181) called *Juglans alba* of MILLER the pignut among other names. MARSHALL in 1785 described *C. cordiformis* as *Juglans alba minima* and called it the pignut; but in 1787 WANGENHEIM called the *Juglans glabra* of MILLER the pignut and *Juglans cordiformis* the bitternut, and these names appear to have been adopted by all later writers on these trees.

CARYA CORDIFORMIS var. LATIFOLIA Sargent, Trees and Shrubs **2**:206. 1913.

New stations for this broad-leaved variety are Fayetteville, Washington County, Arkansas, *E. J. Palmer*, July 15, 1915 (no. 8219), Hannibal, Marion County, Missouri, *J. Davis* (no. 2068), Yellow River and Postville, Allamakee County, Iowa, *O. Schulz*, 1914 (nos. 95 and 30), Toledo, Lucas County, Ohio, *R. E. Horsey*, September 29, 1913.

4. CARYA AQUATICA Nutt.—The water hickory has usually been considered an inhabitant of deep, long inundated river swamps, but toward the southern limits of its range in Florida and Texas it sometimes grows on the high sandy banks of rivers which are only occasionally overflowed, and in dry sandy soil at a considerable distance from streams. The bark of trees in such situations is close, pale, and does not separate into the long, loosely attached scales which are characteristic of this tree when it grows in swamps, although the bark of very old trees growing on dry ground sometimes shows a tendency to flakiness. The trees growing on dry ground have narrower leaflets and usually nuts of a different shape, without the longitudinal wrinkles peculiar to the nut of the water hickory, although some narrower-leaved trees bear nuts of the ordinary size and shape. These southern narrow-leaved trees are so distinct that they may be distinguished as

CARYA AQUATICA var. **australis,** n. var.—Differing from the type in its narrower leaflets, in its smaller ellipsoidal fruit, pale red-brown nuts without longitudinal wrinkles, and in its close pale bark. Leaflets 9–11, lanceolate, acuminate, slightly falcate,

minutely glandular-serrate, the terminal raised on a stem 1–1.5 cm. in length, the lateral nearly sessile; when they unfold scurfy-pubescent above and hoary tomentose below, and in the autumn glabrous on the upper surface, pubescent and furnished on the lower surface with small conspicuous tufts of axillary hairs, 6–8 cm. long and 1–1.5 cm. wide. Fruit ellipsoidal, acute at apex, acute or rounded at base, compressed, covered with small scattered yellow scales, 2–2.5 cm. long, about 1 cm. wide and thick; involucre 1–1.5 mm. in thickness, the sutures only slightly winged, tardily dehiscent usually only to the middle; nut oblong to slightly obovoid or semiorbicular, rounded at the ends, compressed, slightly angled, smooth and without longitudinal wrinkles, the shell 1–1.5 mm. thick.

A large tree with close pale bark.

In dry sandy soil in the yard of a house at Alva, Lee County, Florida, *C. S. Sargent*, March 26, 1914, *T. G. Harbison*, September 16, 1914 (no. 2, type). Banks of the Caloosahatchee River near Alma, common, *C. S. Sargent*, March 26, 1916, *T. G. Harbison*, September 16, 1916. Banks of the St. Johns River, Florida, *A. H. Curtiss*, October (no. 2573), no date.

A tree with close bark growing 5 miles west of Jupiter, Palm Beach County, Florida, with leaflets from 1.5 to nearly 2.5 cm. wide (*C. S. Sargent*, March 19, 1914), is probably of this variety, but I have not seen the fruit. A number of trees with pale close bark on the high banks of the St. Johns River at San Mateo, Putnam County, Florida, seen by Mr. HARBISON and me in 1917, in their rather broader leaflets and larger nuts, resembling in shape the nuts of the typical water hickory but without their peculiar wrinkles, seem to connect the variety with the species. I have seen water hickories with close bark and narrow leaflets growing in dry sandy soil near Marshall, Harrison County, Texas, but the fruit of these trees has not been collected.

5. CARYA MYRISTICAEFORMIS Nutt.—The nutmeg hickory connects the two sections *Apocarya* and *Eucarya* of the genus which without this species might well have been considered distinct genera. The valvate bud-scales and the thin involucre of the fruit with prominently winged sutures show its relationship with *Apocarya*, but the thick shell of the nut and the small number of leaflets, 7–9 and occasionally 5, are characters of *Eucarya*. The lobes of the seed are deeply 2-lobed, like those of *Eucarya*, but this is true of two other species of *Apocarya*, *C. cordiformis* and *C. aquatica*. The seeds of these, however, are bitter, while those of

C. myristicaeformis are sweet and edible, like the seeds of *Eucarya*. Nowhere common and very local in distribution, the nutmeg hickory has recently been found on the bluffs of the Yazoo River near Yazoo City, Mississippi, in Richmond Parish in northern Louisiana, at Natchitoches on the Red River in western Louisiana, at Beaumont on the Nueces River in Texas, and at Hugo in Choctaw County, Oklahoma.

6. CARYA OVATA Koch.—The shagbark hickory shows that little reliance can be placed on pubescence as a specific character in this genus, for individual trees have glabrous or pubescent branchlets and glabrous or pubescent leaflets, the two forms often growing together, so that this variation is not dependent on soil or climate, although pubescent individuals are more common in the south than in the north. On some trees the anthers are red and on others yellow. As it is found from the neighborhood of Montreal in the Province of Quebec to southern Minnesota, *C. ovata* grows farther north than the other species of the genus with the exception of *C. cordiformis*. Westward it ranges to southeastern Nebraska eastern Kansas, and eastern Oklahoma. In North and South Carolina it is confined to the Piedmont region, and on the mountains is replaced by *C. ovalis*. In Georgia I have seen it only on Shell Bluff on the Savannah River, below Augusta, near Eatonton, Putnam County, and at Rome, Floyd County. I have no reason to believe that this tree grows in Florida; and from Alabama I have seen specimens only from Valley Head, Dekalb County, and from the neighborhood of Selma, Dallas County, although MOHR credits it to the "Tennessee valley mountain region upper division of the Coast Pine belt." From Mississippi I have seen specimens only from the east central part of the state (Columbus, Starkville, and Brookville), where it is common in its most pubescent form. It has not been found in eastern Louisiana, but it is common in the western part of that state and in southern Arkansas, but is rare in eastern Texas to the valley of the Trinity River. The common form of the fruit is short-oblong to subglobose and depressed at apex, and the nut is then rounded, truncate, or slightly obcordate at apex. The fruit, however, varies considerably in size and shape, and a small-fruited form has been described as var. *Nutallii*

Sargent, Trees and Shrubs **2**:207. 1913. More distinct is a form with oblong fruit and pointed nuts which may be described as

CARYA OVATA var. **ellipsoidalis,** n. var.—Differing from the type in its ellipsoidal fruit and slender branchlets. Fruit bluntly pointed at the ends, much compressed, slightly angled by winged sutures, rough, 3–3.5 cm. long, about 2.5 cm. wide, and 2 cm. thick; involucre opening to the base, 4–5 mm. thick. Nut ovoid to ellipsoidal, abruptly narrowed at apex into a long acuminate point, rounded at base, more or less compressed and prominently ridged.

Missouri, near Hannibal, Marion County, *J. Davis*, September 5, 1913 (no. 2071, type), Oakwood, Rolles County, *J. Davis*, September 13, 1913 (no. 2132). These trees have unusually slender glabrous reddish branchlets for *C. ovata*, but the foliage is of that species, and the white strongly angled nuts, in spite of their abruptly pointed apex, are clearly the nuts of *C. ovata*. To this variety may be referred a tree found by *James McHugh* at Indian River, Lewis County, New York, September 15, 1911 (no. 11), which differs from the Missouri trees only in its less compressed fruit with an involucre 5–6 mm. in thickness. Nuts of this species more or less pointed at apex are not uncommon, especially at the north, but I have not seen the ellipsoidal compressed fruit with its comparatively thin involucre of this variety except on the specimens from northeastern Missouri and Indian River, New York.

CARYA OVATA var. **complanata,** n. var.—Differing from the type in its broadly obovoid, much compressed, slightly angled nuts cuneate at base, and rounded truncate or slightly obcordate at apex, and in the oblong-obovoid fruit. Fruit 3 cm. long and about 2.5 cm. wide, with an involucre 5–6 mm. in thickness. Nut 2.2–2.5 cm. in length, 2–2.5 cm. in width, and 1.2–1.4 cm. in thickness, with a shell only 1 mm. thick.

A single tree believed to be 50 years old on the Drushel Farm 2 miles southeast of Mt. Hope in Holmes County, Ohio. For *C. ovata* this tree has unusually slender, sparingly villose branchlets and comparatively small villose buds. The leaves are those of the common form of the species. For the specimens of this tree I am indebted to Professor J. ANDREW DRUSHEL, instructor in nature study and geology in the Harris Teachers' College at St. Louis. In the shape of the nuts this is the most unusual form of *C. ovata* which I have seen, and nuts with such a thin shell are not often found in this species.

CARYA OVATA var. FRAXINIFOLIA Sargent, Trees and Shrubs **2**:207. 1913.—This variety, distinguished by its narrow lanceolate leaflets and the rather thinner involucre of the fruit, was based on

specimens collected in western New York. This distinct looking tree is now known to occur in Ohio, Indiana, near Kingston, Ontario, at Keosauqua, Van Buren County, Iowa, and near Myers, Osage County, Oklahoma (*G. W. Stevens*, August 12, 1913, no. 2060).

CARYA OVATA var. **pubescens,** n. var.—Differing from the type in the dense pubescence of pale fascicled hairs on the young branches and on the petioles, rachis, and under surface of the leaflets.

A slight pubescence is not unusual on the branchlets and leaves of *C. ovata*, but this pubescence is sometimes so abundant on individual trees in the southern states that it seems desirable to give them varietal distinction. What I have taken as the type of this variety was collected on May 28, 1918, by *T. G. Harbison* on the bottom lands of the Savannah River at Calhoun Falls, Abbeville County, South Carolina (no. 53). It is a large tree with scaly bark and unusually slender branchlets. The buds are nearly fully grown and are acuminate with the outer scales thickly covered with fascicled hairs. Other specimens referred to this variety are *C. S. Sargent*, banks of Chattanooga Creek, Hamilton County, Tennessee; Valley Head, Dekalb County, Alabama, *T. G. Harbison*, January 26, 1912 (no. 54); *C. Mohr*, Columbus, Lowndes County, Mississippi, October 28, 1898; *T. G. Harbison*, Starkville, Oktibbeha County, and Brookville, Noxubee County, Mississippi, May 3, 1915 (no. 17), October 8, 1915 (nos. 7a, 17a, 26). These trees have slender branchlets and small buds for the species with the exception of those of 17a from Brookville. The pubescence of this tree is rusty brown, but the fruit, which is subglobose and 4.5 cm. in diameter, with an involucre 1.2 cm. in thickness, is clearly that of *C. ovata*.

7. CARYA CAROLINAE-SEPTENTRIONALIS Engl. and Graeb.—New stations for this species are Calhoun Falls, Abbeville County, South Carolina, Columbus, Lowndes County, and Brookville, Noxubee County, eastern Mississippi, and the neighborhood of Selma, Dallas County, Alabama.

8. CARYA LACINIOSA Engl. and Graeb.—The range of this species has been extended to southwestern Ontario, to the valley of the Alabama River near Selma, Dallas County, Alabama, and to West Feliciana Parish, Louisiana (*Cocks*).

9. CARYA ALBA Nutt.—Although this species varies in the size and shape of the fruit and nuts, in the thickness of the branchlets, which are densely covered with fascicled hairs when they first appear, and are pubescent or glabrous in their first winter, and in the size of the winter-buds, which are obtuse or acute, it can always

be recognized by the close slightly ridged dark bark which never becomes flaky, by the dense pubescence on the under surface of the leaflets and on the rachis and petioles, and by the conspicuous reticulate veinlets of the leaflets. The fruit varies from globose to short-oblong, obovoid or ovoid, and is rounded or pointed at apex. The involucre is always thick and usually opens freely nearly to the base by 3 or 4 sutures, the valves generally remaining connected below. The nut is more or less compressed, rounded or acute at base, rounded, acute, or acuminate at apex, slightly ridged usually to the base, and tinged with red; in drying the thick shell often cracks transversely.

This is the common hickory of the south Atlantic and eastern Gulf states, and is always called hickory by the inhabitants of that part of the country, descriptive names being used for the other species. It is less common at the north, and I have not seen specimens from any part of New England north of eastern Massachusetts, from eastern Canada, from Ontario except from the southwestern corner, or from New York west of the Hudson River. It is not rare in Ohio, southern Michigan, Indiana, and Illinois, and becomes very abundant in Missouri and Arkansas; in Florida it is rare except in the northern counties. It is one of the commonest species in Alabama, Mississippi, and Louisiana, and reaches eastern Oklahoma and eastern Texas.

The leaflets, which are usually 7, but occasionally 9, vary much in thickness, and a southern form with very large and thick leaflets has been described as var. *subcoriacea* Sargent, Trees and Shrubs **2**: 207. 1913. This is the common form of southern Arkansas and occurs occasionally from Virginia to Florida, through the Gulf states to eastern Texas, and through Arkansas to Missouri, and has been found in Posey County, Indiana (*C. C. Deam*).

A form of *C. alba* with obovoid fruit, narrowed and rounded above and narrowed below into a stipelike base and compressed nuts acuminate at the ends, has been described as var. *ficoides* Sargent (*l.c.* 206). The type of this variety is in a cemetery at Webb City, Jasper County, Missouri. The same form was collected in 1894 at Ocean Springs, Jackson County, Mississippi, by Josehine Skehan. Nuts of *C. alba* acute or acuminate at apex are not

rare, and trees producing such nuts occur in eastern Pennsylvania and are generally distributed through the southern states. Such pointed nuts are usually acute or acuminate at base and are inclosed in involucres which are oblong, gradually narrowed and rounded at base, and acute or acuminate at apex; but a tree at Noel, Missouri, produces ovoid fruit broad and rounded at base and gradually narrowed and rounded at apex, which is so different from that of other forms of this species which I have seen that it may be distinguished as

CARYA ALBA var. **ovoidea,** n. var.—Differing from the type in its ovoid fruit with a thinner tardily dehiscent involucre. Leaves small for the species, not more than 20 cm. long, with densely pubescent petioles and rachis, and 7 thin leaflets. Fruit smooth and lustrous, not at all compressed, 3.5–4.5 cm. long and 2.5–3 cm. in diameter; involucre not more than 4 mm. in thickness, remaining entirely closed or opening tardily by 2 or 3 of the sutures nearly to the middle. Nut rounded at base, gradually narrowed into a long acuminate apex, irregularly ridged to below the middle, much compressed.

A tree 15 m. high with rough ridged gray bark.

Noel, McDonald County, Missouri, *E. J. Palmer*, September 5, 1913 (no. 4119, fruit, type), October 23, 1910 (no. 3287, leaves).

On the grounds of the P. J. Berckmans Nursery Company, a few miles west of Augusta, Georgia, there is a hickory tree which bears the large oblong acute fruit and acuminate nuts rounded at base of one of those extreme forms of *C. alba* which produce pointed nuts. The bark of this tree is indistinguishable from that of a tree of *C. alba* which is growing close to it. It has glabrous branchlets, however, as slender as those of *C. ovalis*, and acute pubescent terminal winter-buds 8–10 mm. long. The leaves are 7-foliate with thin leaflets and are only slightly pubescent. *C. pallida* grows near this tree, and there are individuals of *C. glabra* not far off. I should have suspected that this tree might be a hybrid between *C. alba* and one of these species, but I can find no trace of either of them in the fruit which is distinctly that of *C. alba*. It may be described as

CARYA ALBA var. **anomala,** n. var.—Differing from the type in its nearly glabrous leaves with smaller leaflets, in its slender glabrous branchlets, and in its smaller winter-buds. Leaves 7-foliate; petioles and rachis only slightly pubescent; leaflets thin, acuminate, finely and remotely dentate, puberulous below and pubescent on

the lower side of the midribs, 14–20 cm. long and 4–6 cm. wide. Fruit oblong, rounded at base, acute at apex, 5 cm. in length; involucre 6 mm. in thickness; nut oblong, rounded at base, acute at apex, compressed, slightly angled to the middle or nearly to the base.

A tree with bark indistinguishable from that of *C. alba* with which it is growing, slender red-brown lustrous glabrous branchlets and acute pubescent terminal buds only 8–10 mm. in length. A single tree on the grounds of the P. J. Berckmans Nursery Company, a few miles west of Augusta, Georgia. This is one of the most abnormal hickory trees I have seen. Without the fruit it might easily be taken for one of the forms of *C. ovalis*, but the fruit and the nuts are clearly those of *C. alba*.

10. **Carya leiodermis,** n. sp.—Leaves 7-, rarely 5-foliolate, 30–35 cm. long with slender petioles and rachis slightly or densely pubescent with fascicled hairs, becoming glabrous or nearly glabrous; leaflets thin, finely serrate, long-pointed at apex, gradually narrowed, cuneate and unsymmetrical at base, the terminal oblong-obovate, raised on a slender pubescent petiolule 1–1.5 cm. in length or nearly sessile, of the same shape and often smaller than those of the upper pair of nearly sessile lateral leaflets; leaflets of the lower pairs lanceolate, petiolulate, much smaller; when they unfold densely covered on the lower surface with hoary tomentum, pubescent above and often ciliate on the margins; fully grown in April when the flowers open, and at maturity dark green and lustrous on the upper surface, pale and slightly pubescent on the lower surface, those of the upper pair 12–15 cm. long and 5–6 cm. wide, with stout midribs more or less densely pubescent on the lower side. Aments of staminate flowers 10–12 cm. long, on slender puberulous peduncles, their bracts lanceolate, puberulous; bracts of the flower ovate, lanceolate, furnished on the margins with long white hairs mixed with stipitate glands, one-third longer than the ciliate calyx lobes; anthers red, covered with long white rigid hairs. Pistillate flowers in short spikes, their involucres and bracts densely clothed with long white hairs. Fruit broadly obovoid, smooth, glabrous or slightly pubescent, sparingly covered with small white scales, 4–4.5 cm. long and 3.5–4 cm. in diameter; involucre 5–5.5 mm. in thickness, opening freely to the base usually by 2 sutures only;

nut oval to slightly obovoid, rounded at the ends, tinged with red, about 3 cm. long and broad and 2.5 cm. thick; shell 4–5 mm. in thickness; seed small and sweet.

A tree 20–25 m. high, with a trunk sometimes 50 cm. in diameter, covered with close only slightly ridged pale bark, and slender reddish brown lustrous branchlets puberulous or pubescent when they first appear, becoming glabrous or almost glabrous by the end of their first season. Terminal winter-buds acute, about 1 cm. long, the outer scales pubescent, the inner covered with appressed pale hairs and ciliate on the margins; axillary buds ovate and rounded at the apex or subglobose.

LOUISIANA: Low woods on Little Bayou Têche 4 miles east of Opelousas, Caddo Parish, *R. S. Cocks* and *C. S. Sargent*, October 11, 1913 (no. 5, type), *R. S. Cocks*, April 1914 (nos. 5, 9, 13, 14); Lake Charles, Calcasieu Parish, *C. S. Sargent*, March 23, 1917; Natchitoches, Natchitoches Parish, *R. S. Cocks*, September 1914 (no. 17), *E. J. Palmer*, April 27, 1915; Grand Ecore, Natchitoches Parish, *E. J. Palmer*, April 28, May 5, 1915 (nos. 7411, 7412, 7524), April 15, 1916 (no. 9452); dry woods, Winnfield, Winn Parish, *Cocks* and *Sargent*, April 6, 1913; Tangipahoa Parish, *Cocks* and *Sargent*, March 28, 1917, roadside between Springfield and Ponchatoula, *Sargent* and *Cocks*, March 29, 1917, Loranger, *Sargent* and *Cocks*, March 30, 1917.

ARKANSAS: Fulton, Hempstead County, *E. J. Palmer*, October 18, 1915 (no. 8953).

MISSISSIPPI: Bluffs, Yazoo City, Yazoo County, *T. G. Harbison*, May 1 and 30, 1915 (nos. 22, 36, 37), October 26 and 28, 1916 (nos. 46, 48).

The slender often glabrous branchlets of *C. leiodermis* show its relationship with *C. ovalis;* the close bark, the thick involucre of the fruit, the reddish nut, and the pale tomentum on the under surface of the young leaflets indicate a connection with *C. alba*, and its proper position seems to be between these two species.

CARYA LEIODERMIS, var. **callicoma,** n. var.—Differing from the type in the thinner involucre of the fruit and in the bright red color of the unfolding leaves. Leaves 7-foliate, the young leaflets coated below with hoary tomentum, ciliate on the margins and scurfy-pubescent above, bright red and very fragrant, and at maturity thin, dark green and lustrous on the upper surface and yellow-green and nearly glabrous on the lower surface. Fruit slightly obovoid, smooth, nearly glabrous, usually 3–3.5 cm. long or rarely not more than 2 cm. long; involucre 2.5–3 mm. in thickness, opening tardily nearly to the base by 2 or 3 sutures; nut rounded at the ends, compressed, only slightly angled, pale brown, 1.5–2.5 cm. long and wide, the shell 3.5 mm. in thickness.

A tree 25–30 m. in height, with a tall trunk sometimes 1 m. in diameter, covered with close gray-brown ridged but not scaly bark, ascending and spreading branches forming a narrow round-topped head, and slender glabrous red-brown branchlets marked by numerous pale lenticels.

LOUISIANA: Low woods, borders of streams and river banks often overflowed; Lake Charles, Calcasieu Parish, *C. S. Sargent*, April 2 and 3, 1913, April 12, 1914, *R. S. Cocks*, October 1913, September 1914, *R. S. Cocks* and *C. S. Sargent*, April 12, 1915; West Lake Charles, *R. S. Cocks* and *C. S. Sargent*, April 1913.

TEXAS: Low woods near Beaumont, *C. S. Sargent*, April 11, 1915, *E. J. Palmer*, April 22 and September 11, 1916 (nos. 9528, 9532, 10694, 10695).

MISSISSIPPI: Vicksburg, Warren County, *T. G. Harbison*, October 28, 1916 (no. 3); near Natchez, Adams County, *Miss C. C. Compton*, May 1915; Jackson, Hinds County, *T. G. Harbison*, April 29, 1915; Taylor, Lafayette County, *T. G. Harbison*, April 14, 1915 (no. 7); Rockport, Copiah County, *T. G. Harbison*, 1915, 1916, 1917 (nos. 2, 3, 15, 16, 17); Columbus, Lowndes County, *C. S. Sargent*, October 12, 1914; Starkville, Oktibbeha County, *T. G. Harbison*, April and October, 1913 (nos. 1059, 1283).

From other hickories this variety differs in the bright red color of the young foliage, which in early spring makes it one of the most distinct and beautiful trees in the forests in the neighborhood of Lake Charles, where it is common. It may be expected to occur generally in the region between Lake Charles and the valley of the lower Nueces River, Texas.

11. **Carya pallida,** nov. comb.—*Hicoria pallida* Ashe, Notes on hickories. 1896.—This tree is closely related to *Carya ovalis* Sargent, but may be distinguished from all the forms of that species by the pale under surface of the leaflets, by the silvery scales on the young foliage, and by the prominent and persistent clusters of fascicled hairs on the petiole, rachis, and under side of the midrib. The leaves are 7-, rarely 9-foliate, and vary from 1.5 to 6 cm. in width. The fruit is pubescent and varies from ellipsoidal to obovoid or to broad-obovoid and to subglobose or depressed globose, and from 2 to 4 cm. in length, and is not easily distinguishable from that of some forms of *C. ovalis*. The involucre varies from 3 to 4 mm. in thickness and splits tardily to the base, usually by 2 or 3 of the sutures. The nut is white, rounded at the ends or occasionally slightly obcordate or obtusely pointed at apex, compressed and more or less prominently ridged nearly to the base. On a tree growing on the grounds of the Country Club at Summerville, near Augusta, Georgia, the fruits are pyriform, 5 cm. long, and contracted below into a stipelike base with an involucre 5 mm.

thick and oblong much compressed nuts narrowed at apex. The branchlets of *C. pallida* are slender, glabrous or pubescent, and the winter-buds are acute or obtuse, and are covered with yellow scales. When *C. pallida* grows in rich soil it sometimes attains a height of 30–35 m. and forms a trunk 6 m. in diameter. On such trees the bark is pale and only slightly furrowed. On dry stony ridges trees more than 10–15 m. tall are not common, and the bark of trees growing in such soil is sometimes nearly black, very rough with prominent ridges.

Carya pallida grows in New Jersey in sandy soil in the neighborhood of Cape May. It is common in sandy soil in southern Delaware and in the southern part of the Maryland peninsula. It is common in Gloucester and James City counties, Virginia, where it often grows in rich soil and attains its largest size. It occurs in Isle of Wight County, Virginia, and is common in the Piedmont region of North and South Carolina, and in the western parts of these states ascends into mountain valleys up to elevations of 700 m. above the sea-level. It is common in northern and central Georgia, and occasionally reaches the Georgia coast. It grows at Bainbridge, southwestern Georgia, and in Leon and Gadsden counties, Florida. In Alabama it is the common hickory on the dry gravelly and poor soil of the upland table lands and ridges of the central part of the states, and extends into the southwestern counties. The western stations from which I have seen specimens of this tree are Chattanooga, Tennessee, Yazoo City, Mississippi, where it is common on the bluffs of the Yazoo River, and northeastern Louisiana (near Kentwood, Tangipahoa Parish, *Cocks*).

Trees of this hickory can easily be recognized at a distance by the pale color of the under surface of the leaves, and southward by the dark, deeply fissured bark of the trunk, which is not found on other hickories in the southeastern states. Formerly I considered that *C. pallida* was the same as *C. villosa* from Allenton, Missouri, and other authors have adopted this view, but further observations show that it can be distinguished from that tree by the absence of the rusty brown pubescence from the unfolding leaves and young branchlets, by the silvery scales on the young leaves, by the pale color of the under surface of the leaflets, and by the thicker involucre of the larger often ellipsoidal or globose fruit.

12. CARYA GLABRA Sweet.—The name pignut, which should be confined to *Carya cordiformis*, has been generally applied to many trees with smooth or slightly scaly bark, slender branchlets, small winter-buds, and pear-shaped or globose fruit. The husk of the fruit of these trees varies in thickness; it remains closed or opens

only at the apex or splits to the base; it is puberulous and, like the involucre of the pistillate flower, is covered more or less thickly with yellow scales, which are usually found also on the lower surface of the 5 or more commonly 7 leaflets. The different forms of these trees intergrade, and it would be possible to consider them forms of one species; but as the trees with close bark usually produce pear-shaped fruits which remain closed or open tardily to the middle, and generally by only 1 or 2 sutures, and as the trees with scaly bark bear fruit which, although round or pear-shaped on different forms, always splits freely to the base, it seems convenient to group these different forms under two species, chiefly distinguished by the indehiscent or dehiscent involucre of the fruit. The earliest post-Linnaean name for any of these trees is *Juglans glabra* of MILLER, published in the eighth edition of his *Dictionary* in 1768. MILLER'S species is based on CLAYTON'S *Juglans alba fructu minori, cortice glabro*. Trees with close bark and indehiscent pear-shaped fruit and trees with slightly scaly bark and globose dehiscent fruit are common in Gloucester County, Virginia, where CLAYTON lived for many years and where he probably made most of his observations on trees, but the "cortice glabro" seems to point to the tree with close bark and pear-shaped indehiscent fruit. If this view is correct and the trees with indehiscent fruit are treated as representatives of a distinct species, this becomes

Carya glabra Sweet, Hort. Brit. 97. 1827; Torrey, Fl. N.Y. **2**:182 (in part). *pl. 101.*

Juglans glabra Miller, Dict. ed. 8, no. 5. 1768.

Juglans porcina Michaux f., Hist. Arb. Am. Sept. **1**:206 (in part). *pl. 38. figs. 1, 2.* 1910.

Carya porcina Nuttall, Gen. **2**:222 (in part); Sargent, Trees and Shrubs **2**:199. *pl. 179.* 1913.

The fruit of this tree is obovoid, compressed, rounded at apex, gradually narrowed below and often abruptly contracted into a short stipelike base; the involucre is 1.5–2.5 mm. in thickness and opens tardily generally by 1 or 2 sutures, or often remains closed. The nut is compressed, obovoid, slightly obcordate or acute at apex, gradually narrowed at base, not ridged, and light colored with a hard thick shell and small sweet seed. The leaves are usually 5-, rarely 7-foliolate, and more or less thickly covered with yellow scales. The bark is close and shows no tendency to become flaky. This is one of the least

common of the forms of the so-called pignut, but ranges from southwestern Vermont to western New York and southeastern Ontario (Queensland Heights), and southward to Delaware, the District of Columbia, eastern Virginia, and along the Appalachian Mountains to North Carolina; it occurs in northern, central, and eastern Georgia, northern Alabama, eastern Mississippi, and in southern Indiana, where it is common, and in southeastern Illinois (*H. A. Gleason* in Herb. Gray). MICHAUX's figure on which SWEET based his *Carya glabra* represents a rather longer fruit than that of the common form of this tree and the involucre has opened by 4 sutures nearly to the middle of the fruit. *Carya glabra* passes into

CARYA GLABRA var. **megacarpa,** nov. comb.—*Carya megacarpa* Sargent, Trees and Shrubs **2**:201. *pl. 180.* 1913; *Carya ovalis* var. *megacarpa* Ashe, Torreya **18**:74. 1918.—Differing from the type in its larger fruit with a thicker involucre and in its usually stouter branchlets and larger winter-buds.

This tree has larger fruit with a thicker involucre, usually stouter branches, larger buds, and close bark which shows little tendency to become flaky. The thickness of the branchlets, the size of the buds, and the size of the fruit, however, cannot always be depended upon to distinguish this form, for some of the southern trees which bear the largest fruit have branchlets as slender as the northern small-fruited trees; and trees with stout branchlets sometimes produce as small nuts as the small-fruited form of *C. glabra.* This variety is usually glabrous, but on a tree in Rochester, New York, and on one at Brunswick, Georgia, the leaves are distinctly pubescent. The fruit varies from 2.5 to 4.5 cm. in length, with an involucre 2.5–3 mm. in thickness and is occasionally entirely covered with bright yellow scales; it varies from oblong-obovate, with a distinctly stipelike base, to short-obovate and rounded at base, or to subglobose. The nut is rounded or acute at the ends. The leaves are 5–7-foliate.

In the north this form has been seen only near Rochester, New York, on the New Jersey Coast, in the District of Columbia, and in southern Illinois; it is one of the most abundant hickories in the coast region of the southeastern states from North Carolina to the Florida peninsula, and to Alabama, where it is a common tree on the shores of Mobile Bay, and Louisiana. It ranges occasionally inland to central and northern Georgia and to western Mississippi.

CARYA GLABRA var. MEGACARPA f. **angulata,** n. f.—Differing from the type in the striately angled nuts. The fruit of this form is broadly obovoid, depressed at apex, 2.5–3 cm. long, 2.8–3 cm. wide, and about 2.5 cm. thick; the involucre is 3–4 mm. in thickness and remains closed after the fruit is perfectly dry. The nut is

subglobose, but rather broader than high, and conspicuously angled to the base, with a shell 4–5 mm. in thickness. The leaves of this form are 5–7, usually 7-foliolate. It is a tree with wide spreading branches, pale gray shallowly grooved close bark, slender glabrous bright red-brown lustrous branchlets, and acute winter-buds, the terminal 5–8 mm. long, their outer scales covered with gray pubescence.

Borders of salt marshes, near Brunswick, Glynn County, Georgia, *T. G. Harbison*, March 26 and October 1, 1913, November 13, 1914 (nos. 1024 type, 1025, 1027, 1028).

13. CARYA OVALIS (Wangenheim) Sargent, Trees and Shrubs **2**:207. 1913.—This is the oldest name which can be used for the small-fruited hickories with globose or pear-shaped fruit opening usually as soon as ripe to the base generally by the 4 sutures of the thin involucre, and often with slightly scaly bark. The type of this tree and its varieties have glabrous or rarely slightly pubescent leaves, with usually 7 thin leaflets. The type of the species, judging by WANGENHEIM'S figure, has short-oblong fruit rounded at base, acute at apex, 2.5–3 cm. long and about 1.5 cm. in diameter, with an involucre 2–2.5 mm. in thickness. This is one of the least common of the forms of this tree, and occurs from western NewYork and eastern Pennsylvania to Illinois, and southward to the mountains of North Carolina and Tennessee, and to central Georgia and Alabama. The following varieties can be distinguished:

CARYA OVALIS var. OBCORDATA Sargent, *l.c.* 208. 1913, with subglobose to short-oblong fruit 2–3 cm. in diameter. The bark often separates into narrow scales, but on some trees shows no tendency to become scaly.

This is the commonest and the most widely distributed of the northern forms of this tree and the *Carya* or *Hicoria microcarpa* of many recent authors. It is common in southern New England and ranges to Wisconsin, southwestern Missouri, western North Carolina, central and eastern Georgia, eastern Mississippi, and to central Alabama, where it is very common in the mountain districts. On the Carolina mountains this tree grows to a large size and is sometimes called scaly-barked hickory. On dry ridges in Macon County, North Carolina, and near Birmingham, Alabama, the bark is close and darker, and some of the trees look distinct from the red color of the petioles which they retain during the season.

CARYA OVALIS var. OBCORDATA, f. **vestita,** n. f.—Differing from the var. *obcordata* in the thick tomentose covering of the branchlets during their first year. The leaflets of this form are slightly pubescent in the autumn on the under surface of the midribs. Although the nuts are more compressed than those of the ordinary forms of var. *obcordata*, the fruit is of that variety. The branchlets are unusually stout for a form of *C. ovalis* and are covered with rusty tomentum during their first year and are more or less pubescent in their second and third seasons.

A large tree on the low border of Davis Pond 14.3 miles southwest of Decker, Knox County, Indiana, *C. C. Deam*, October 5, 1917 (no. 24, 144 type).

CARYA OVALIS var. ODORATA Sargent, *l.c.* 1913.—The fruit of this form shows less tendency to vary than that of the other forms and is subglobose or slightly longer than broad, much flattened and 1.3–1.5 cm. in diameter, with an involucre often not more than 1 mm. thick. The bark of old trees is often scaly.

This form is not common, but ranges from southern New England, eastern Pennsylvania, and the District of Columbia to western New York, Ohio, Indiana, southeastern Ontario, and southern Illinois. From the southern states I have seen specimens only from the neighborhood of Atlanta, Georgia, and from Starkville, Oktibbeha County, Mississisppi.

CARYA OVALIS var. BOREALIS Sargent, *l.c.* 1913.—This variety differs from *C. ovalis* in its pubescent branchlets and winter-buds and in the pubescence on the leaves early in the season. It has ellipsoidal or ovoid flattened fruit with an involucre 3–3.5 mm. thick and an ovoid nut conspicuously ridged to the base. The bark is scaly.

This variety has only been noticed in southwestern Michigan.

CARYA OVALIS var. OBOVALIS Sargent, *l.c.* 1913.—This form has more or less obovoid fruit about 2.5 cm. long and 2 cm. in diameter. The fruit resembles in shape that of *Carya glabra*, but the involucre is thicker and splits easily to the base or nearly to the base.

This form is found from southern New England to Missouri and northern Arkansas, and occurs on the mountains of North Carolina, on the coast of Georgia, and in northern central Alabama, and is the common "pignut" in the middle western states.

CARYA OVALIS var. OBOVALIS f. **acuta,** nov. f.—*Carya porcina* var. *acuta* Sargent, Trees and Shrubs **2**:200. *pl. 179. figs. 9, 10.* 1913.—In spite of its close bark this tree seems to belong with *C. ovalis* rather than with *C. glabra*. The bark and the nuts pointed at the ends afford the only characters by which it can be distinguished from *C. ovalis* var. *obovalis*.

CARYA OVALIS var. **hirsuta,** nov. comb.—*Hicoria glabra hirsuta* Ashe, Notes on hickories. 1896.—This is a common tree on the southern Appalachian Mountains of North Carolina at elevations of from 1200–1500 m. above the sea, and occasionally grows to a height of 20–25 m. with a trunk diameter of 6 m. The scaly bark of this tree shows its relationship with *Carya ovalis* rather than with *C. glabra*, and I have taken up ASHE's name, although the petioles and lower surface of the leaflets are not tomentose as he describes them, but pubescent, the fascicled hairs which are more or less abundant on different individuals being most numerous on the under side of the midribs. The fruit is pyriform, usually narrowed below into a short stipitate base, 3–4.5 cm. in length, more or less compressed, with an involucre tardily dehiscent, usually opening only to the middle, and 1.5–3 mm. in thickness. The nut is compressed, very slightly ridged, and rounded at the ends, with a thin shell and a sweet seed. The winter-buds are pubescent, acute or obtuse, the terminal varying from 7 to 14 mm. in length.

Highlands, Macon County, North Carolina, *T. G. Harbison*, 1913 and 1914 (no. 1). *Harbison* no. 1250, June and October 1914, with less pubescence and slightly obovoid fruit, with a thin involucre splitting freely to the base and a slightly obovoid nut, appears to be a form connecting the variety with the species.

14. CARYA FLORIDANA Sargent, Trees and Shrubs **2**:193. *pl. 177.* 1913.—When I described this species I had not seen terminal winter-buds and I mistook it for an *Apocarya*. Collections made later show that the terminal winter-buds are ovate, acute or obtuse, and 5–7 mm. long, and that the scales are imbricated and covered with close rusty pubescence and more or less thickly with yellow or rarely silvery scales. The branchlets are glabrous or pubescent during their first winter. Later collections show that the fruit is obovoid, gradually narrowed, rounded, and sometimes slightly

depressed at apex, narrowed below into a short stipelike base, occasionally slightly winged at the sutures, sometimes roughened by prominent reticulate ridges, puberulous and covered with small yellow scales, 2–3.5 cm. long and 2–2.5 cm. in diameter; the involucre is 2–3 mm. in thickness, splitting to the base by usually 2 or 3 sutures. The nut is pale or reddish, subglobose, and not more than 1.5 cm. in diameter, or ovate, acute at base, narrowed and rounded at apex, slightly compressed, or rarely oblong and acute at base, rounded at apex, and 2.5–3 cm. long and 2 cm. wide; the shell varies from 2–3 mm. in thickness; the cotyledons are sweet.

Although the fruit and thin branchlets of *C. floridana* resemble those of the *glabra-ovoidea* group, the thick rusty pubescence on the young leaves and branchlets separates it from all the plants of this group. It differs from them also and from other hickories in the occasional appearance of the aments of staminate flowers from the axils of leaves, and in the fact that it is often shrubby in habit and produces large crops of fruit on stems not more than 2–3 m. high. The sessile, or nearly sessile, terminal leaflet is also unusual in the genus.

Carya floridana is common on the eastern coast of Florida, growing on dry sandy ridges and low hills from Valusia County southward to Jupiter Island, Palm Beach County. It is common, too, usually as a small shrub near Orlando in Orange County and southward to De Soto County, and occurs on the shore of Pensacola Bay.

THE TEXAS HICKORY.—In 1860 BUCKLEY described his *Carya texana* in Proc. Philad. Acad. As the name was otherwise occupied DURAND changed it to *C. Buckleyi*, and as BUCKLEY described the fruit as globose with a thin involucre *C. Buckleyi* has been adopted for a tree with globose fruit, a thin involucre, and pale red nearly globose nuts. This tree with the round nuts is common in the neighborhood of Denison, Grayson County; it grows also near Jacksonville in Cherokee County, at San Augustine, San Augustine County, and at North Pleasanton, Atascosa County, and in Oklahoma on dry sandy hills west of Muskogee, Muskogee County.

The hickory with obovoid or ovoid fruit, often with an involucre varying greatly in thickness, and with an oblong or slightly obovoid compressed slightly angled pale nut, which I described as *C. arkansana* (Trees and Shrubs **2**:203. *pl. 181*), is much more common and more widely distributed in Texas, and it is probable that BUCKLEY

had the 2 trees in mind when he described his *C. texana*, for when they grow together in dry sterile soil, as in the neighborhood of Denison, they both have thick nearly black fissured bark and cannot be distinguished except by the shape of the fruit. Farther north and in better soil the bark of *C. arkansana* is thinner, lighter-colored, and is inclined to separate into small thin scales. The unfolding leaves and the young branchlets of both trees are thickly covered with tawny pubescence mixed on the under side of the leaflets with small silvery scales. This pubescence distinguishes them and *C. floridana* from all the other species of the United States. The size and shape of the fruit and the thickness of the involucre do not afford good specific characters in *Carya*, and the nature of the bark is so dependent within certain limits on the soil in which the individual grows that this cannot be depended upon for distinguishing species. The winter-buds on both trees are covered with brownish pubescence in which silvery scales are more or less scattered; and the long white hairs found at the apex of the scales of the former sometimes occur but are perhaps more often wanting from the scales of the latter. The thick tawny pubescence is the most distinct and constant character of all the forms of this tree. The form with obovoid fruit can perhaps best be treated as a variety which becomes

15. CARYA BUCKLEYI var. **arkansana,** nov. var.—*C. arkansana* Sargent, Trees and Shrubs **2**:203. *pl. 181.* 1913.—Differing from the type in the obovoid to ellipsoidal or ovoid fruit with a usually thicker involucre, and in the oblong more compressed pale-colored nuts.

The type region of this tree is the valley of the Arkansas River at Van Buren, near Fort Smith, in the extreme western part of the state of Arkansas. It has been found growing in sandy soil near Vollmer, Knox County, Indiana (*C. C. Deam*, no. 18232, August 28, 1915), and it is common in northeastern Missouri, where it has been collected by the Reverend JOHN DAVIS at a number of stations near Hannibal. It is the common hickory on the Ozark Mountains in northwestern Arkansas, where it is very abundant on dry rocky ridges at elevations of 400–600 m., and occurs in several other parts of Missouri and in Arkansas and eastern Oklahoma. It is not rare in western Louisiana, where it has been collected in the neighborhood of Opelousas, at Winnfield, and near Alexandria. In Texas it is the common hickory from the coast to the base

of the Edwards Plateau and as far south as the valley of the Atascosa River in Atascosa County, where it was first collected by BUCKLEY in June 1881. From farther northwest I have seen specimens only from Fredericksburg in Gillespie County. As in other species of the genus, there is considerable variation in different individuals. The fruit is obovoid, rounded or gradually narrowed at the base or abruptly contracted into a more or less developed stipe, or ellipsoidal or ovoid and rounded at the ends; it varies from 2 cm. to 5 cm. in length and in diameter. The involucre varies from 2 mm. to 4 mm. in thickness, the largest fruit with the thickest involucre being found in southern Arkansas and western Louisiana, and the smallest in northern Missouri. The nuts are oblong to slightly obovoid, compressed and rounded at the ends and vary much in size but little in shape or in the thickness of the shell, which is unusually thick for a species of this group. The thickness of the branchlets, which are pubescent during the first year, and the size of the winter-buds vary on different trees. The leaflets as they unfold are covered above by small scattered yellow scales and on the lower surface are thickly clothed with thicker tawny scales mixed with silvery white scales, and are pubescent on the midribs and veins, traces of these fascicled hairs being persistent during the season. The scales and fascicled hairs are also found on the young petioles and rachis, which usually become quite glabrous before the end of the season. The yellow scales, sometimes mixed with short hairs, are more or less persistent on the fruit and on the winter-buds.

CARYA BUCKLEYI var. ARKANSANA, f. **pachylemma,** n. f.—Differing from the var. *arkansana* in its larger fruit with a thicker involucre. The fruit of this form is 5–6 cm. long and 4–5 cm. in diameter with an involucre 1.2–1.3 cm. in thickness; the nut is rounded at the ends, slightly angled, compressed, from 3.2 to 3.5 cm. long and about 3 cm. wide.

A large tree with thick deeply fissured pale gray bark, small drooping unusually slender nearly glabrous branchlets and rusty pubescent winter-buds.

Rich woods, Fulton, Hempstead County, Arkansas, *E. J. Palmer*, April 27, 1914 (no 5396), October 19, 1914 (no. 6878), April 10 and 12, 1915 (nos. 7172, 7184), June 17, 1915 (no. 8032), October 18, 1915 (no. 8952).

This tree, which in the size and thickness of the involucre produces remarkable fruit, long puzzled Mr. PALMER and me until the unfolding leaves showed its relationship with *Carya Buckleyi*.

A hickory tree which is common and widely distributed in Missouri and northwestern Arkansas has the peculiar rusty brown pubescence of the Texas hickory on its young leaves and branchlets and on its winter-buds, and although the fruit is smaller this tree

cannot be specifically distinguished from that species, and is here treated as

CARYA BUCKLEYI var. **villosa,** nov. comb.—*Hicoria glabra* var. *villosa* Sargent, Silva N. Am. **7**:167. *pl. 355.* 1895; *Hicoria villosa* Ashe, Bull. Torr. Bot. Club **24**:481. 1897; Sargent, Man. 145 (in part). 1903; *Carya villosa* Schneider, Ill. Handb. Laubholz. **1**:803. 1906; *Carya glabra* var. *villosa* Robinson, Rhodora **10**:32. 1908.—Differing from the type in its smaller obovoid or ellipsoidal fruit with a thinner often indehiscent involucre.

A single tree, the type of this variety, was found nearly 40 years ago by the late GEORGE W. LETTERMAN on a dry rocky hillside at Allenton, St. Louis County, Missouri, where it is still growing. LETTERMAN considered it a hybrid. Before the Texas hickory and its varieties were recognized it was considered a variety of *Carya glabra*, from which it differs in its pubescence and in its usually more dehiscent involucre. There would be more reason in following ASHE and treating it as a species did not trees occur with fruit which approaches in its larger size and thicker involucre that of the var. *arkansana* which occasionally grows with it in Missouri.

The Allenton tree has thick, rough, deeply furrowed, nearly black bark similar to that of *C. Buckleyi* as it grows near Denison, Texas. On trees growing in better soil in other parts of the state the bark is often paler and less deeply furrowed. The leaves of the typical tree are 5–7-foliolate, with pubescent petioles and rachis, becoming glabrous or nearly glabrous; the leaflets are lanceolate to oblanceolate, long-pointed, with prominent reticulate veinlets, the lateral nearly sessile, the terminal short-petiolulate, nearly glabrous above and early in the season covered below with rusty pubescence and small brownish scales, in the autumn glabrous or nearly glabrous with the exception of the fascicled hairs on the lower side of the midrib. The fruit of the Allenton tree is obovoid, cylindrical, sometimes slightly winged above the middle, about 2.5 cm. long and 1.8 cm. in diameter, rusty pubescent and covered with scattered yellow scales; the involucre is about 2 mm. in thickness and is indehiscent or splits tardily to the base usually only by 2 sutures. The nut is ovoid, rounded at base, pointed at apex, only slightly angled, thin-shelled, and faintly tinged with red. The

branchlets are slender, pubescent during their first year and puberulous in their second season. The winter-buds are covered with rusty brown pubescence and yellow scales, and often furnished near the apex with the tufts of white hairs, which are generally found on the buds of *Carya Buckleyi*. The fruits on trees in other parts of the state vary from obovoid to ellipsoidal, or are rarely subglobose; they vary from 1.5 to 3 cm. in length, and are nearly cylindrical or much compressed; the involucre varies from 1 to 4 mm. in thickness, and on some trees the fruit is completely covered by the yellow scales. On some trees the branchlets lose their pubescence early and by the end of September are glabrous, red, and lustrous.

I have seen specimens of this tree collected in Missouri by the Reverend *John Davis* in the neighborhood of Hannibal, Marion County (nos. 1361, 1630, 2028, 2032, 2078, 2089, 2156, 2160, 2162, 2163, 2166, 2182, 2188, 2190, 2237); in Grain Valley, Jackson County, *B. F. Bush*, May 24, 1913 (nos. 6981, 6991); at Jerome, Phelps County, *J. H. Kellogg*, May 7, 1913 (nos. 333, 339, 340, 341, 347, 348, 357); Allenton, St. Louis County, *G. W. Letterman*, June 20, 1880, May 15, 1881, May 1, 1882, April 1883, July 16, 1911, May 10, 1912, *J. H. Kellogg*, October 7, 1911, *E. J. Palmer*, August 13, 1917 (no. 12652); Des Arc, Iron County, *E. J. Palmer*, July 2, 1914 (no. 6165); Branson, Tenney County, *E. J. Palmer*, October 23, 1913 (no. 4707), June 18, 1914 (no. 5891); Willow Springs, Howell County, *E. J. Palmer*, July 8, 1914 (no. 6227); dry hillsides near Campbell, Osage County, *C. S. Sargent*, September 5, 1915; dry barren hills, Joplin, Jasper County, *E. J. Palmer*, October 1911 (no. 3494), May 17 and September 18, 1913 (nos. 3491, 3928, 4356, 4357, 4358, the last with ellipsoidal fruit on a long peduncle, 4359, 6890, 6891); Noel, McDonald County, *E. J. Palmer*, September 6 and 14, 1913 (nos. 4060, 4159, 4170, 4216, 4336, 4337, 5410).

ARKANSAS: Eureka Springs, Carroll County, *E. J. Palmer*, September 22 and 24, 1913 (nos. 4428, 4482), May 11, 1914 (no. 5548).

HYBRID HICKORIES.—The supposed hybrids between species of *Apocarya* are

CARYA BROWNII Sargent, Trees and Shrubs **2**:195. *pl. 178.* 1913. —This tree grows on the bottom lands of the Arkansas River below Van Buren, Crawford County, Arkansas. In the Arboretum Collection are nuts of what is no doubt the same hybrid collected at Collinsville, Rogers County, Oklahoma. To this hybrid probably belongs the so-called Galloway hickory (see S. GALLOWAY in Gar-

dening **2**:26. 1874; TRELEASE in Rep. Mo. Bot. Gard. **7**:33. *pl. 16. figs. 15, 16. pl. 20;* Sargent, *l.c.* 196).

A tree evidently of the same parentage has been described as

CARYA BROWNII var. VARIANS Sargent, Trees and Shrubs **2**:196. 1913.—This tree grows on the banks of Sears Creek near the Pump House of the Van Buren Water Works, Crawford County, Arkansas. A tree with similar fruit has been found near Natchez, Adams County, Mississippi, by Miss C. C. COMPTON.

Three evident hybrids between species of *Apocarya* and *Eucarya* are known.

CARYA LANEYI Sargent, Trees and Shrubs **2**:196. 1913.—This appears to be a hybrid of *C. cordiformis* and *C. ovata*. The original tree is in the Riverside Cemetery at Rochester, New York. In the Arboretum Collection there are nuts of a tree growing at Millersville, Lancaster County, Pennsylvania, which is known as the Beaver hybrid and appears to be of the same parentage. Trees of the same parentage but with the leaves of *C. cordiformis* and with larger fruits with thicker involucres than those of that species and nuts resembling those of *C. ovata* have been distinguished as

CARYA LANEYI var. CHATEAUGAYENSIS Sargent, *l.c.* 1913.—First discovered at Chateaugay near the mouth of the Chateaugay River in the Province of Quebec by Professor J. G. JACK, this tree was later found by him at Summertown, Ontario.

Carya Schneckii, n. hyb. (*C. alba*×*pecan*).—Leaves 7–9-foliolate, glabrous; leaflets thin, acuminate at apex, cuneate and unsymmetrical at base, falcate, short-petiolulate. Fruit oblong, acute at apex, rounded at base, pubescent, 5.5 cm. long, with an involucre splitting to the base and 6–7 mm. in thickness; nut oblong, gradually narrowed and rounded at base, acuminate at apex, slightly compressed, angled to the middle or to the base, reddish and conspicuously streaked with brown, 4–4.5 cm. long and about 2 cm. wide, with a shell 1–2 mm. in thickness and a sweet kernel.

A large tree with bark resembling that of the pecan, stout reddish brown puberulous branchlets, and winter-buds with imbricated scales, the outer dark red-brown and puberulous, the inner thickly covered with hoary tomentum; axillary buds solitary with usually valvate scales.

Lawrenceville, Lawrence County, Illinois, *Dr. J. Schneck*, October 15, 1895 (type) (see Sargent, Silva N. Am. **7**:138). A tree believed to have been of the same parentage was found at about the same time by Mr. F. REPPERT near Muscatine, Muscatine County, Iowa (see TRELEASE in Rep. Mo. Bot. Gard. **7**:39. *pl. 23. figs. 2–5*).

Carya Nussbaumerii, n. hyb. (*Carya laciniosa*×*pecan*).—Leaves 7–9-foliolate; petioles and rachis puberulous; leaflets lanceolate, long-pointed and acuminate at apex, rounded and unsymmetrical at base, pubescent on the lower surface, the terminal petiolulate, the lateral nearly sessile. Fruit oblong, narrowed and rounded at base, acute at apex, puberulous and more or less thickly covered with yellow scales, about 7 mm. long and 3.5–3.8 cm. in diameter; involucre splitting nearly to the base and 4 mm. in thickness. Nut oblong, compressed, only slightly angled, short-pointed at apex, rounded at base, 6 mm. long, 3.5 cm. wide, and 2.5 cm. thick, with a shell 1.5–2 mm. in thickness.

I suggest this name for the Nussbaumer hybrid (see SARGENT, Silva N. Am. **7**:158. *pl. 349. fig. 4;* TRELEASE in Rep. Mo. Bot. Gard. **7**:41. *pls. 22, 23. figs. 7–9*).

This tree was first found on the bottoms between Mascoutah and Fayetteville, St. Clair County, Illinois. A tree producing a similar nut which came originally from Illinois was cultivated before 1892 by Mr. R. M. FLOYD of Cedar Rapids, Iowa, and has been called the Floyd nut. In October 1895 Dr. SCHNECK found a tree producing similar fruit at Mt. Vernon, Posey County, Indiana. Grafted plants of this tree, which has been called the McCallister (see Nut culture in the United States, Bull. U.S. Dept. Agric., Div. of Pomology, 1896, 63. *pl. 9. fig. 6*), were sent to Washington, Georgia, whence this tree was distributed as the Washington nut, a name now abandoned by pomologists. Another of these trees has been reported from the neighborhood of Burlington, Des Moines County, Iowa, and another, known as the Rockville nut, from near Rockville, Bates County, Missouri. In its foliage and in the color of the branchlets this hybrid resembles *C. laciniosa*. The branchlets, however, are not as stout and are less pubescent than those of that species, and the buds are smaller and more acuminate. The fruit in shape resembles that of the pecan, but does not have the sutural wings of that species, and the nut is white or nearly white and only slightly streaked with brown.

Carya Dunbarii, n. hyb. (*C. laciniosa*×*ovata*).—I suggest this name for a number of trees found growing on the bottoms of the Genesee River at Golah, Monroe County, and Mount Morris,

Livingston County, New York, by JOHN DUNBAR, assistant superintendent of the parks of the city of Rochester, New York. These trees, which have at different times been considered both *C. laciniosa* and *C. ovata*, vary among themselves in the color and pubescence of the branchlets, in the size of the buds, and in the size and shape of the fruit and nuts. The leaves have the 7 or 9 leaflets of *C. laciniosa*, but the leaflets are usually narrower than those of that species and less pubescent. I have selected no. 68 *Dunbar*, September 19, 1911, as the type of this hybrid, as the tree is still standing and can easily be located.

Leaves 7-foliate, the petioles and rachis slender, glabrous; leaflets acuminate, puberulous on the lower surface and pubescent on the under side of the midribs, the terminal oblong-obovate, cuneate, and gradually narrowed below into a slender petiolule 1.5 cm. in length, the lateral lanceolate to oblanceolate, nearly sessile. Fruit oblong, rounded at ends, glabrous, 4 cm. long, 3 cm. in diameter; involucre splitting to the base, 5 mm. in thickness; nut oblong, gradually narrowed and rounded at base, acute at apex, compressed, conspicuously ridged to below the middle, pale brown, 3 cm. long, 2.5 cm. wide, and 2 cm. thick.

A tree 80–90 ft. high, with light gray scaly bark, stout spreading branchlets puberulous early in the season, glabrous and pale red-brown in the autumn. Terminal buds, oblong, acute, 1.5–1.8 cm. long and 6–7 mm. in diameter, the outer scales dark red-brown and puberulous.

Golah, Monroe County, New York, *J. Dunbar*, September 19, 1911 (no. 68, type).

No. 71 Golah, collected by *J. Dunbar* September 19, 1911, has the leaves in the size and shape of the leaflets like those of *C. ovata;* the petioles and rachis are pubescent, and the leaflets are more pubescent than those of no. 68. The fruit is oblong-obovoid, compressed, rounded at base, abruptly acute at apex, 4 cm. long, 3 cm. wide, and 2 cm. thick, with an involucre splitting nearly to the base and 4 cm. thick. The nut is oblong-obovoid, acute at the ends, only slightly angled, and pale in color.

This tree, which is still standing, is 80 ft. high, with ashy gray bark divided into plates but not separating into loosely attached scales like that of its supposed parents, and stout reddish glabrous

branchlets. The terminal winter-buds are acute, 1.8 cm. long, the outer scales pale pubescent, the inner hoary tomentose. In the number of leaflets the leaves of this tree resemble those of *C. laciniosa*, but in the shape of the leaflets they resemble those of *C. ovata* var. *fraxinifolia*. In shape the fruit resembles a small fruit of *C. laciniosa*, but the involucre is thinner than that of *C. ovata* or *C. laciniosa*. The nut is more like that of *C. laciniosa;* the bark and color of the branchlets are unlike those of either of the supposed parents. The winter-buds are more like those of *C. laciniosa* than of *C. ovata*.

No. 61 Golah, *J. Dunbar*, September 29, 1911, has the leaves of *C. laciniosa*, short-oblong fruit 4 cm. long, depressed at the apex like that of *C. ovata*, with an involucre 6 mm. in thickness. The branchlets are red and glabrous and unlike those of either parent. Except in the branchlets this number resembles a small-fruited *C. laciniosa*.

No. 66 Golah, *J. Dunbar*, September 19, 1911, has the leaves of *C. laciniosa*, oblong slightly obovoid pubescent fruit only 3 cm. long, with an involucre 4 cm. in thickness, and a compressed slightly angled reddish nut. The branchlets are reddish, pubescent, and about as thick as those of the common form of *C. ovata*. The buds are acute and 7–8 mm. long, with puberulous outer scales.

No. 73 Golah, *J. Dunbar*, September 19, 1911, has the leaves of *C. laciniosa*, fruit similar to that of no. 66 and slender, densely pubescent brown branchlets resembling those of a pubescent form of *C. ovata*.

No. 207 Golah, *J. Dunbar*, September 19, 1911, has the leaves of no. 68, fruit like no. 61, stout glabrous red branchlets and terminal buds 1.5 cm. long, the outer scales covered with pale pubescence.

No. 208 Golah, *J. Dunbar*, September 19, 1911, has leaves resembling in shape those of *C. ovata*, fruit like that of no. 73 and 3 cm. long; branchlets somewhat stouter and less pubescent than those of no. 73, and the winter-buds of *C. ovata*.

No. 250 Golah, *J. Dunbar*, August 31, 1915, has the leaves of *C. laciniosa*, obovoid pubescent fruit 3–3.5 cm. long, with an involucre 5 mm. in thickness, and a slightly compressed angled

nut. The branchlets resemble those of the pubescent form of *C. ovata*.

No. 251 Golah, *J. Dunbar*, August 31, 1915, has only slightly pubescent leaves with leaflets resembling in shape those of *C. ovata* var. *fraxinifolia*. The fruit is pubescent, subglobose, 3 cm. long and rather broader than long, with an involucre 3 mm. in thickness; the nut, although less prominently ridged, resembles the nut of *C. ovata*. The branchlets are reddish brown and puberulous.

No. 252 Golah, *J. Dunbar*, August 31, 1915, has the leaves of *C. laciniosa*, oblong pubescent fruit 3 cm. long, with an involucre 5 mm. in thickness and conspicuously angled nuts. The branchlets are stout, dark red-brown, and densely pubescent. The terminal bud is about 1 cm. in length. Except in the color of the branchlets, the small size of the buds, and in the small size of the fruit, this number resembles *C. laciniosa*.

No. 253, *J. Dunbar*, August 31, 1915. Although only puberulous, the leaves otherwise generally resemble those of *C. laciniosa*. The fruit is similar to that of no. 73, but the involucre is 7 mm. in thickness; the nut is only slightly compressed and angled. The branchlets are reddish brown, pubescent, and as stout as those of the common form of *C. ovata*. The bud is 1 cm. long with pubescent outer scales.

No. 254 Golah, *J. Dunbar*, August 31, 1915, has leaves resembling those of no. 68, the fruit of *C. laciniosa*, and red nearly glabrous winter branchlets. The winter-buds are 1 cm. long with puberulous outer scales.

No. 59, Mount Morris, Livingston County, *J. Dunbar*. This has leaves like no. 68 from Golah; the fruit is that of *C. laciniosa* and 4 cm. long. The branchlets are slender, reddish, and glabrous; the winter-buds are about 1 cm. in length with pubescent outer scales.

There is so much variation in these trees that their hybrid origin seems probable. The most remarkable things about them are the red glabrous lustrous branchlets of some of the trees; these are entirely unlike those of either of the supposed parents and suggest that one of the forms of *C. ovalis* or *C. glabra* might have had some influence on them. If they are hybrids in large part between

C. laciniosa and *C. ovata*, as I believe, they are the only hybrids between two species of *Eucarya* which have been noticed, other hybrids of *Carya* having been produced by the crossing of 2 species of *Apocarya* or of a species of *Apocarya* with a species of *Eucarya*. In the case of other hybrids of *Carya* only a single tree or single trees in different locations have been noticed. The hickory trees in western New York, however, have been more carefully examined by Mr. DUNBAR and his associates than the hickories in any other part of the United States. When the trees in other parts of the country are as carefully and intelligently studied, it is possible that many hybrid hickories and many individuals of these hybrids will be found, just as in recent years many hybrid oaks often with numerous individuals have been found.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

FERTILIZATION IN LILIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 243

WANDA WENIGER

(WITH PLATES XI–XIII)

Introduction

The cytological phenomena of fertilization have been studied with greater detail in the gymnosperms than in the angiosperms. This paper is the result of an attempt to discover whether there is a similarity between the process of fertilization as already described for gymnosperms and that of angiosperms. *Lilium* has long been the type used in the study of fertilization in the classroom; it was chosen for the subject of study in this case because it lends itself particularly well to cytological work. The writer is indebted to Dr. C. J. CHAMBERLAIN for the suggestion of the problem and for his helpful assistance throughout the progress of the work.

In *Pinus* (**1, 3, 5, 7, 8**), *Tsuga* (**14**), *Juniperus* (**18, 19**), and *Abies* (**12**) evidence has been brought to bear upon the fact that no fusion of the male and female chromatic substance takes place.

BLACKMAN (**1**), in 1898, described the cytological features of fertilization in *Pinus silvestris*. While the outlines of the 2 sexual nuclei are still visible, the chromosomes are found in 2 separate clumps; and even on the spindle fibers of the first division they can be distinguished into 2 groups. After a longitudinal splitting the half chromosomes fuse together in the telophase of the division.

CHAMBERLAIN'S (**3**) account of oogenesis in *Pinus Laricio* includes figures of the male and egg nuclei. He states that after the male pronucleus is within the oosphere nucleus the chromatin of the 2 pronuclei appears as 2 distinct masses in the spireme stage. "Perhaps segmentation of the 2 spiremes occurs while they are still separate." In *Tsuga canadensis* MURRILL (**14**) reports 2 sets of chromosomes distinct in the equatorial region of the first spindle.

Miss FERGUSON (7), in her first paper on *Pinus Strobus* in 1901, finds that the 2 chromatic groups are distinctly recognizable at the time of the segmentation of the spiremes, and can still be clearly made out during the early development of the chromosomes, but not as late as the equatorial plate stage. "There is never any fusion, as ordinarily understood, of the male and female nuclei." In her second paper on *Pinus* Miss FERGUSON (8) describes the longitudinal splitting of the 24 chromosomes on the equatorial plate. According to NORÉN (18, 19), the essential features of fertilization in *Juniperus communis* are similar to those of *Pinus*.

A very detailed account of fertilization is given by HUTCHINSON (12) for *Abies balsamea*. Two groups of chromatin at the micropylar end of the egg nucleus, one male and the other female, become separated into 16 chromosomes each, and these pass on to the spindle fibers. The 2 spindles in the metaphase fuse, and the chromosomes are arranged to form 16 pairs, each pair forming a C, in which the 2 chromosomes are twisted about each other. By means of a transverse break at the angle of the bent chromosomes each pair forms 4 segments. Of the 64 segments, 32 go to each pole, where in the daughter nuclei they remain very distinct.

CHAMBERLAIN counted 12 chromosomes in *Stangeria* (4) at the equatorial plate stage of the division of the fertilized egg, while the sporophyte number is 24. He accounts for the haploid number by assuming the chromosomes to be of a double character, and supports HUTCHINSON'S view of the pairing of chromosomes.

In angiosperms the behavior of the chromatin during fertilization has received little attention. In the majority of cases the statement is made that the nuclei fuse while in the resting condition almost immediately after they come in contact and form a definite resting nucleus, differing only in its greater size from the unfertilized egg nucleus.

GUIGNARD'S (9) paper in 1891 on fertilization in *Lilium Martagon* contains statements overlooked by most writers. The formation of 2 distinct spiremes in the male and egg nuclei was observed but not figured. No fusion is brought about between the chromatin of the 2 nuclei, even when the nuclear membranes disappear. The segments of each spireme pass on to the equatorial plate, where

each splits longitudinally. In 1895 MOTTIER (**13**) first described the vermiform shape of the male nuclei in *Lilium Martagon*. The male and egg nuclei fuse in the resting condition after coming in contact and are figured as forming a resting nucleus. In 1898 NAWASCHIN (**15**) announced the discovery of double fertilization in *Lilium Martagon* and *Fritillaria tenella*. The male nucleus that fuses with the polar nuclei loses its spiral form, but the 3 nuclei remain distinct until the prophase of the division. The fusion of the 3 nuclei occurs when the numerous chromosomes come together on the equatorial plate. "Fusion occurs, not in the resting stage, as MOTTIER indicates, but in the prophases of the division, as GUIGNARD first observed."

The motility of the male nuclei is described for *Lilium Martagon* and *Fritillaria tenella* by NAWASCHIN (**16, 17**); for the tulip by GUIGNARD (**11**); and for *Lilium Martagon* and *L. auratum* by BLACKMAN and WELSFORD (**2**), and Miss WELSFORD (**23**). These authors attribute independent motion to the male nuclei.

In *Paris quadrifolia* and *Trillium grandiflorum* ERNST (**6**) finds a striking difference between the fusion of the male nucleus with the egg and that with the polar nuclei. In the former case the fusion is complete, so that a typical resting nucleus is formed. In the latter case the polar nuclei begin to form spiremes even before the male nucleus arrives, and in the group of the 3 nuclei (the 2 polar nuclei and the male nucleus) 3 spiremes are distinguishable.

Distinct maternal and paternal chromosomes were first described for an angiosperm by Miss PACE (**21**). She found spiremes in all nuclei of the embryo sac of *Cypripedium* before fusion took place. The spireme was well formed in every nucleus, and shortened almost enough to segment into chromosomes. "It would seem in this case, that if fusion does take place, there could be no possibility of a fusion of the chromatin, which would certainly divide into chromosomes from the spireme as it is now formed."

NAWASCHIN (**17**) published another paper on *Lilium Martagon* in 1910, again emphasizing the fact that the mature nuclei are capable of movement. He finds that the mitosis of the 2 male nuclei in the pollen tube is characterized at an early stage by sharply differentiated chromosomes, so that the sperm nuclei do not reach

the resting stage, but remain in the condition characteristic of a telophase.

Recently SAX (**22**) has investigated *Fritillaria pudica*. In most cases it is not until the male nucleus and the egg nucleus have completely fused that he finds any appearance of the formation of the spireme. In rare cases, however, the spireme stage is found while the 2 nuclei are still distinct in outline. He believes that the rare appearance of such cases is probably of little significance, since it is probable that these nuclei subsequently fuse completely because no later stages were found in which fusion was incomplete. From the many stages and abundant cases of triple fusion he observed he thinks there is no doubt that the 2 polar nuclei and the male nucleus fuse completely and that the subsequent division is normal.

Methods

Stages in fertilization were obtained from ovaries of *Lilium philadelphicum* collected in the field near Osborn, Calumet, and Pine, Indiana, in June and early July, 1916, at the time when the petals "snapped," and after the petals had fallen. To correlate the time of pollination with stages in fertilization, flowers were brought into the laboratory, pollinated, and kept under bell jars for several days, until fertilization had taken place. In general, it may be said that the petals drop on the third day after pollination, and the style separates from the ovary on the fourth or fifth day. The male nucleus was in contact with the egg nucleus from 60 to 72 hours after pollination.

The material for *Lilium longiflorum* was obtained from plants grown in the greenhouse. It produced seeds readily, although it is generally reported not to set seed. The male nucleus was in contact with the egg nucleus about 120 hours after pollination. Of the upward of 500 cases of fertilization observed in these 2 species, the majority showed the male and egg nuclei in contact, with their chromatin in early prophases of the division.

Chrom-acetic-osmic and Flemming's medium solutions were used as fixatives, and the ovaries trimmed so as to permit more rapid penetration of the embryo sacs. Sections were cut 10 μ thick and stained with Flemming's triple stain or Haidenhain's iron-alum-haematoxylin.

Observations

Upon leaving the pollen tube the male nuclei retain their coiled shape for some time. The egg nucleus (fig. 1), with chromatin in a resting condition before the arrival of the male nucleus, remains in this condition, while the male nucleus lies in contact with it. Stages can be found abundantly in which the male nucleus has penetrated the egg and lies adjacent to the egg nucleus, and in which the chromatin of the former is in an early prophase (fig. 2), or spireme stage (fig. 3), while the chromatin of the latter more lightly staining nucleus is in the resting stage. The male nucleus is more or less curved around one side of the egg nucleus and usually measures about 9 μ at its short diameter, while the spherical egg nucleus is 10–12 μ in diameter. Soon the male nucleus becomes more rounded, as is shown in fig 4, where the chromatin in both nuclei is still in the same stage as in fig. 3.

The chromatin of the egg nucleus is then formed into a spireme (figs. 5, 6); but this spireme was never found to stain as densely or become as regular as that of the male nucleus. The membranes of the 2 nuclei seem still to be in contact at this stage. No fusion of the spiremes takes place, but each is segmented into chromosomes independently. This account agrees with that of GUIGNARD for *Lilium Martagon*, where no fusion takes place between the chromatic elements of the 2 nuclei.

In the gymnosperms investigated the separate groups of chromosomes formed from the male and female spiremes respectively become oriented on separate spindles, and then the 2 spindles fuse during the metaphase. Whether or not this is true for *Lilium* has not been determined. The entire process of fertilization in *Lilium* is an exceedingly rapid one, since the time elapsing between the discharge of the male nuclei and the formation of the 2-celled embryo is probably not longer than 8 hours. Since the contact stage of the 2 nuclei in the prophases of the division is of such relatively common occurrence in preparations made, it would seem that it occupies the greater part of this time, and that for this reason the actual division of the fertilized egg is a very difficult stage to obtain. One very favorable preparation shows this division, with some of the chromosomes still on the equatorial plate and others already

near the poles of the spindle. Figs. 11, 12, and 13 represent the 3 sections of this spindle, and in fig. 14 the 3 drawings are superimposed and slightly diagrammed. Of the 24 chromosomes present on the equatorial plate, 12 are contributed by the male nucleus and 12 by the egg nucleus. The chromosomes are not drawn into the sharp U's and C's so characteristic of divisions in *Lilium*. The chromosomes come together in pairs in which they twist more or less about each other (fig. 12*a*). Each of the chromosomes of the 12 pairs then breaks transversely at the center of the ellipse it forms, each pair giving rise to 4 segments. The 48 segments in the form of small rods remain paired (fig. 12*b*, *c*) as they move toward the poles of the spindle. The components of each pair are similar in size so far as could be determined; one segment is male and the other female in origin. In fig. 14 the 12 pairs of chromosomes are represented, with the 4 segments of a pair indicated by the same number. All segments going to one pole are in black, those to the opposite pole in outline. Chromosomes 8 and 12 have not as yet come in contact and the transverse break has not yet appeared. This behavior of chromosomes resembles that of the first reduction division in tetrad formation. There is a pairing of chromosomes and a subsequent transverse breaking. The result of the division is not the reduced number of chromosomes, however, but the diploid number, for only a transverse break occurs, and no further splitting.

In the telophase of this division (figs. 15, 16) no further evidence of the pairing of the chromosomes could be observed. It would seem probable that the individuality of the chromosomes derived from the male and egg nuclei would persist. The second division (fig. 17) of the fertilized egg is in all respects like the ordinary vegetative division in *Lilium*, with a longitudinal splitting of the characteristic U-shaped chromosomes during the metaphase.

Observations were also made on the behavior of the chromatin during triple fusion. The process occurs much more rapidly and the resulting nucleus divides at least twice before the fertilized egg undergoes division. At the time that the endosperm nucleus divides (fig. 7) the male and egg nuclei are still in the stage shown in fig. 3 or 4. The 2 polar nuclei, with membranes distinct, are in the resting condition when the male nucleus in the spireme stage comes

in contact with them (fig. 8). A spireme is then formed in each polar nucleus also (fig. 9), and the nuclear membranes disappear at the point of contact of the nuclei. The lower polar nucleus is usually a little larger than the one coming from the micropylar end of the embryo sac. The male nucleus is at the left in fig. 9.

Segmentation of spiremes occurs so that on the spindle (fig. 19) the chromosomes are extremely long and U-shaped. The number of segments is difficult to ascertain, but it approaches the $3x$ number. The division is accomplished by a longitudinal splitting of chromosomes, producing in the anaphase a mass of long bent segments that cannot be counted with any satisfaction.

There is a striking difference between the first division of the fertilized egg and that of the endosperm nucleus. The former is characterized by shorter straighter chromosomes, a pairing of chromosomes, and a subsequent transverse breaking of each pair to form 4 segments, of which 2 go to each pole. The division of the endosperm nucleus resembles the ordinary vegetative division by means of a longitudinal splitting of chromosomes. The number of chromosomes is $3x$. Since previous cytological work has not covered the necessary phases, it is possible that the description of the behavior of chromosomes during the first division of the fertilized egg here given may apply quite generally to angiosperms. A longitudinal splitting of chromosomes on the equatorial plate would bring about the same result in that the $2x$ number of chromosomes goes to each of the daughter nuclei; but the supposition of a longitudinal splitting would not account for the situation described. If a longitudinal splitting should occur before the transverse breaking, rather than a pairing of chromosomes, the resulting number would be 96 rather than 48 segments.

The 3 phases of fertilization, union of cells, union of nuclei, and union of chromosomes, occur in rapid succession in animals, since the reduction division immediately precedes fertilization. In plants the 3 processes may be separated for a longer or shorter period. In the rusts there is a long gap between the union of the gametes at the base of the aecidium and the nuclear and chromosome conjugation. In some of the green algae, such as *Oedogonium*, the 3 come close together, since reduction follows soon after fertilization. In the brown algae the 3 are also close together, but reduction

precedes fertilization. In the higher plants cell and nuclear union have been thought to come close together at the beginning of the sporophyte generation, while the chromosome union did not occur until during the reduction division at the end of the sporophyte generation. In *Abies*, as found by HUTCHINSON; in *Stangeria*, according to CHAMBERLAIN; and in *Lilium* there seems to be evidence of a chromosome union at the time of fertilization.

Summary

1. The egg nucleus is in a resting condition when the male nucleus, in spireme stage, comes in contact with it.

2. Distinct male and female spiremes are formed which are segmented into chromosomes while the nuclei are in contact.

3. On the equatorial plate the male and female chromosomes come together in x number of pairs and divide by means of a transverse break, each pair forming 4 segments. The segments move to the poles in pairs. Of the $4x$ segments formed, $2x$ go to each pole of the spindle.

4. The chromosomes on the equatorial plate of the second division of the fertilized egg divide longitudinally.

5. The endosperm nucleus divides at least twice before the fertilized egg undergoes division.

6. A distinct spireme is formed in each of the nuclei of the triple fusion, and the $3x$ segments are oriented on the equatorial plate.

7. The endosperm nucleus divides in the typical vegetative manner by means of a longitudinal splitting of the chromosomes.

EXPERIMENT STATION
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EXPLANATION OF PLATES XI–XIII

All drawings were made with an Abbé camera lucida at table level and Zeiss apochromatic objectives and compensating oculars. For fig. 7 the 8 ocular and 2.0 mm. objective were used, giving a magnification of 1500; for the remainder of the drawings the 18 ocular was used with the 2.0 mm. objective, and the magnification was 4000. All drawings were reduced one-half in reproduction.

PLATE XI

FIG. 1.—Egg before fertilization, with nucleus in resting condition; ×2000; *L. philadelphicum.*

FIG. 2.—Male nucleus in early prophase; egg nucleus in resting condition; ×2000; *L. philadelphicum.*

FIG. 3.—Chromatin of egg nucleus more irregular; male nucleus still curved around egg nucleus; ×2000; *L. philadelphicum.*

FIG. 4.—Male nucleus rounded; ×2000; *L. longiflorum.*

FIG. 5.—Early spireme in egg nucleus; ×2000; *L. philadelphicum.*

FIG. 6.—Distinct spiremes in male and egg nucleus; segmentation begun; ×2000; *L. longiflorum.*

FIG. 7.—Endosperm nucleus undergoing division while egg nucleus is in resting stage and male nucleus in contact with it shows a spireme; ×750; *L. philadelphicum.*

PLATE XII

FIG. 8.—Triple fusion: male nucleus in spireme stage, upper and lower polar nuclei with chromatin in resting stage; ×2000; *L. longiflorum.*

FIG. 9.—Distinct spiremes in 3 nuclei of triple fusion; male nucleus at upper left; ×2000; *L. philadelphicum.*

FIG. 10.—Metaphase of endosperm nucleus; ×2000; *L. philadelphicum.*

FIG. 17.—Second division of fertilized egg; ×2000; *L. longiflorum.*

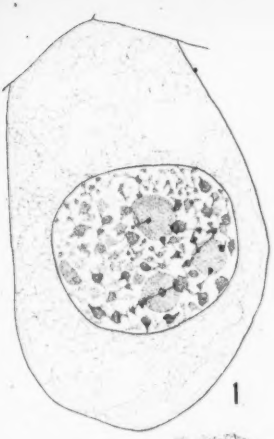
PLATE XIII

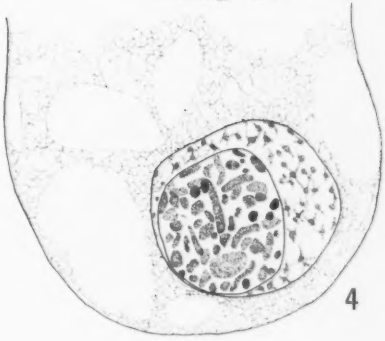
FIGS. 11–13.—Three sections of spindle of fertilized egg in division, showing pairing of chromosomes, transverse break, moving of pairs to the poles; ×2000; *L. longiflorum.*

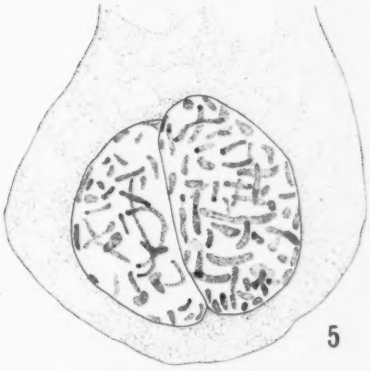
FIG. 14.—Diagram of division of fertilized egg, made by superimposing figs. 11–13; the 12 pairs of chromosomes are represented, with 4 segments of a pair indicated by the same number; segments in solid black go to one pole, while those in outline go to the other pole; chromosomes numbered 8 and 12 have not yet paired or segmented.

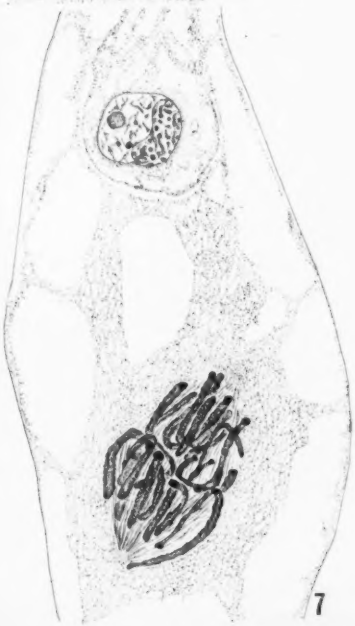
FIG. 15.—Early telophase of first division; ×2000; *L. longiflorum.*

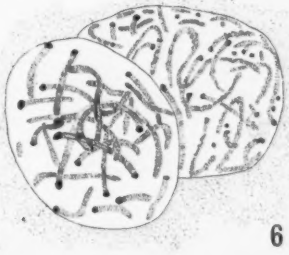
FIG. 16.—Late telophase of first division; ×2000; *L. longiflorum.*











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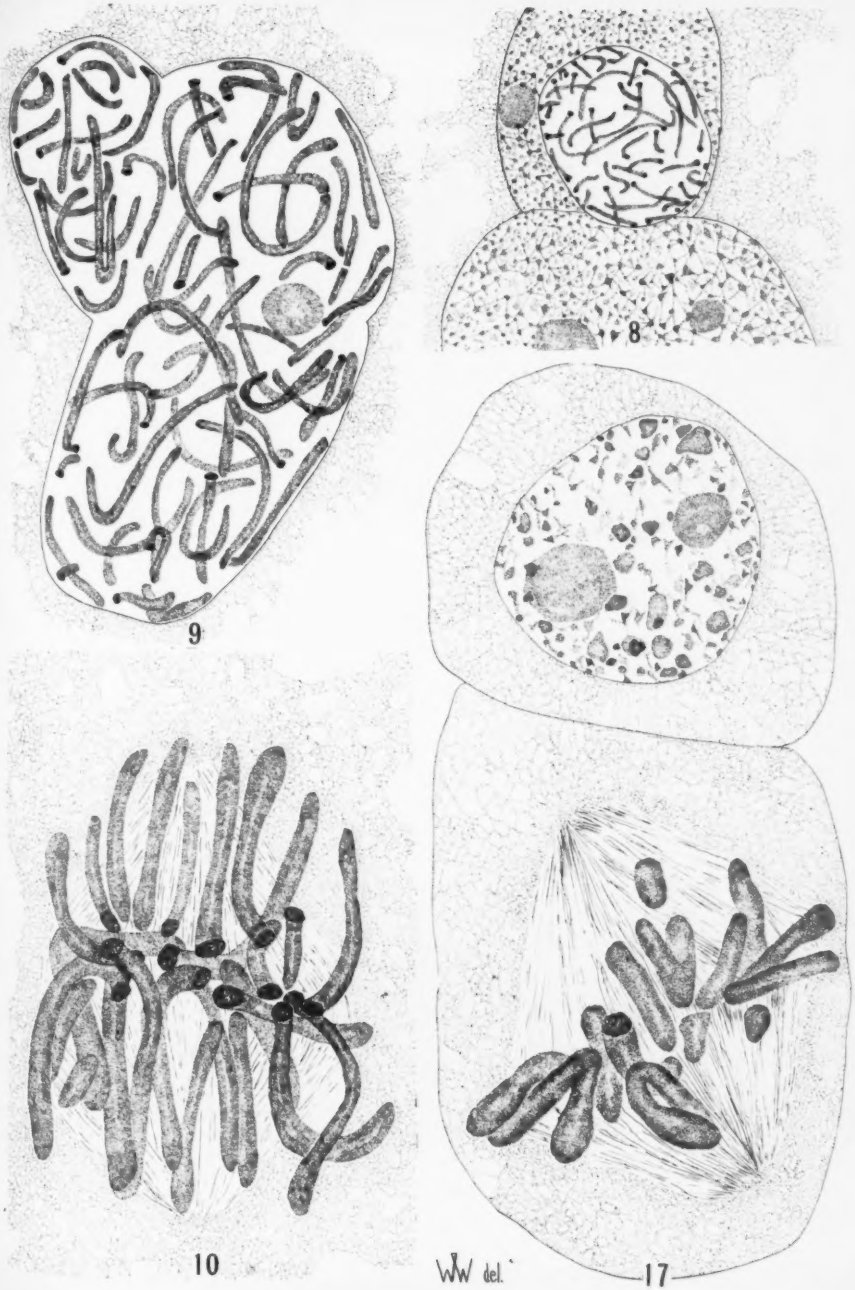
WENIGER on LILIUM



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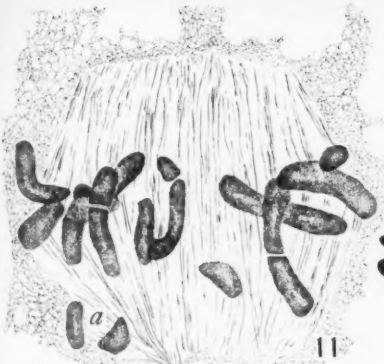

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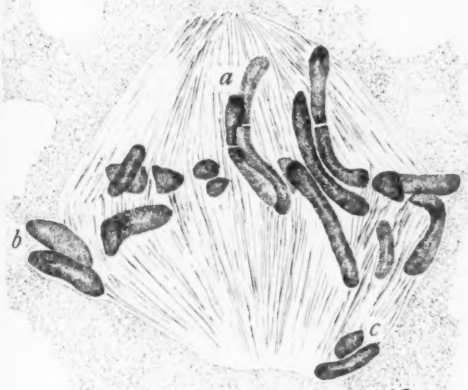

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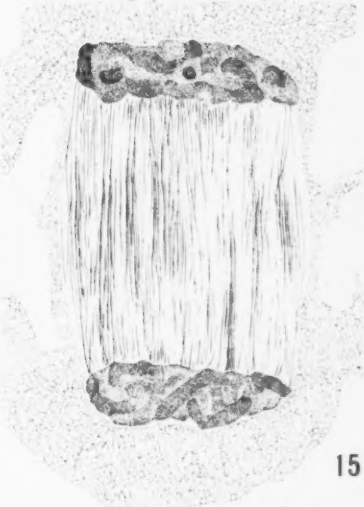

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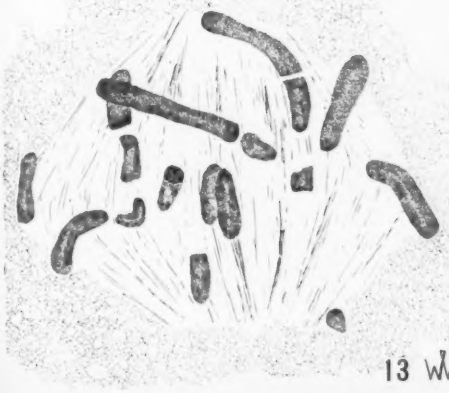

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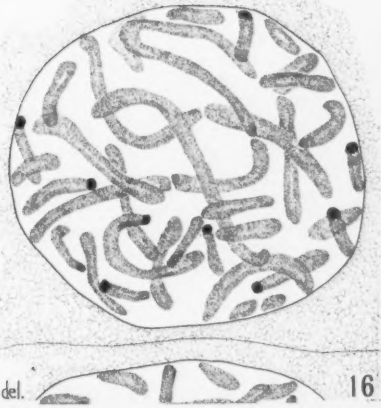

16

WENIGER on LILIUM

ABNORMAL CONJUGATION IN SPIROGYRA

J. G. BROWN

(WITH THREE FIGURES)

Recently while teaching a class in plant histology, the attention of the writer was directed by one of his students to the conjugating cells of *Spirogyra* shown in the accompanying figures. The material from which the figures were drawn, which answered to WOLLE's

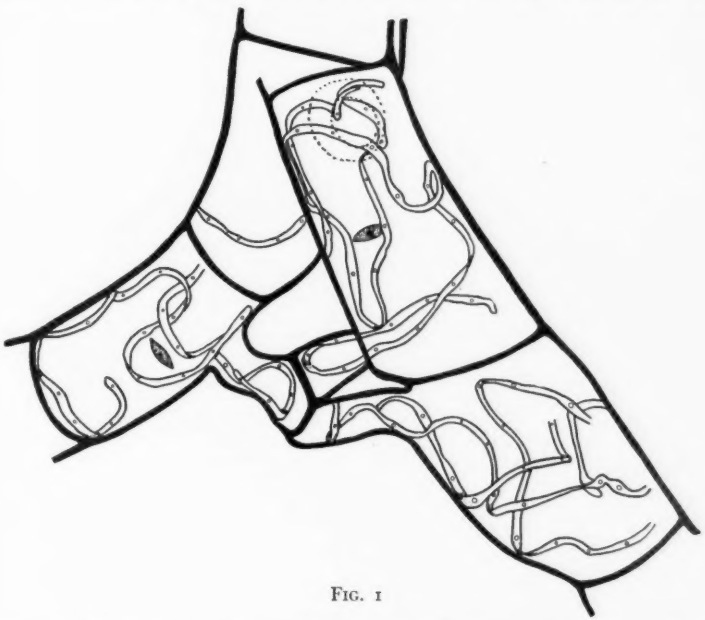

FIG. 1

description of *S. nitida*,[1] was collected in the Rillito River north of Tucson in April 1917. When they were examined under low power lens, the conjugating cells shown in fig. 1 presented the appearance of a knot. Upon analyzing the situation, one of the four cells was found to have connections with three others, two with

[1] WOLLE, FRANCIS, Fresh-water algae of the United States, p. 217.

two others, and the fourth with one other. Three of the conjugation branches formed a triple connection, and two other cases of triple connection were found on the same slide. The cells represented in fig. 2 also presented an interesting study in reproduction. One cell was here monopolizing the energies of two cells in an adjacent filament, while its neighbor on each side had resorted to parthenogenesis. Although several slides were examined, no cases of lateral conjugation were observed.

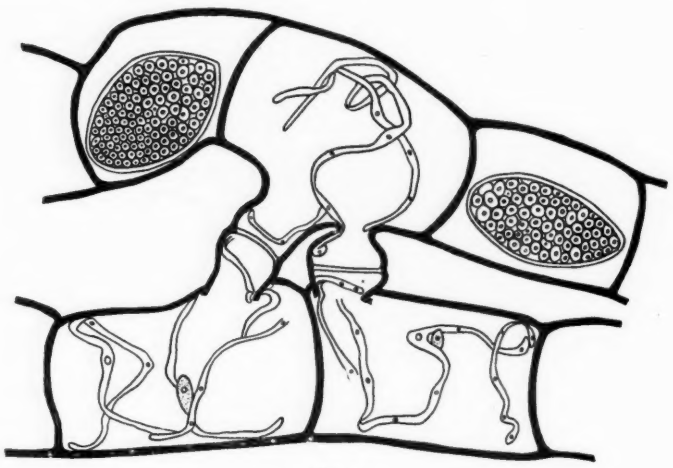

FIG. 2

Abnormal conjugation in other species of *Spirogyra* has been mentioned by several investigators, notably by the WESTS,[2] who have examined material from many different countries. Several types of scalariform conjugation between three cells have been described: (*a*) by means of four branches connecting three cells belonging to two different filaments; (*b*) by means of four branches connecting three cells belonging to as many different filaments; (*c*) by means of three branches forming a triple connection. According to the WESTS the last type mentioned is very rare. They illustrate a case of triple connection of conjugation branches in *S. condensata* in which one of the three branches has prevented the

[2] WEST, W. and G. S., Observations on the Conjugatae. Ann. Botany 12:29–58. 1898.

protoplasts of two of the three cells concerned from fusing. This appears to be a common result, and the large proportion of failures has been interpreted as proving the abnormality of the method. Another illustration included in the paper cited represents a condition in *S. maxima* similar to the one described in this note in fig. 2 for *S. nitida*, excepting the parthenogenetically formed spores. The great profusion of conjugation branches exhibited occasionally by *Spirogyra* filaments, accompanied by the tendency to form abnormal connections, has been regarded as a response to environmental conditions exceptionally unfavorable for vegetative growth. In this region such external factors as the volume,

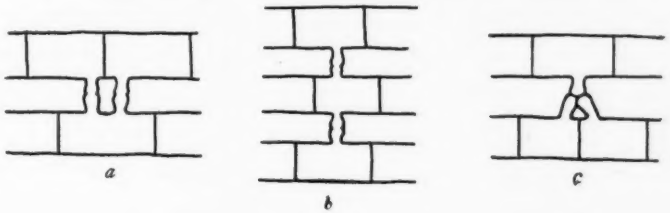

FIG. 3

temperature, and salt content of water are extremely variable. The fluctuation in water volume may be such that in a few days a large, rapidly flowing stream is changed to a trickling brook, then to a series of stagnant pools, then later to a "dry river" carrying its entire flow beneath the surface of the bed. In the winter snow water reaches the foothill and mesa country in a cold condition after showers in the mountains. Floods of this cold fresh water must have a decided influence on the algal vegetation of pools by lowering the temperature and salt concentration, increasing aeration, and thus making the vegetative conditions more favorable. Subsequent evaporation and the "run-off" from local showers increase the salt content to a maximum and again subject algae to unfavorable vegetative conditions, thus bringing on great reproductive activity. The *Spirogyra* figured in this note was collected in a pool which had gone through a similar cycle of changing conditions.

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BRIEFER ARTICLES

CROSS-CONJUGATION IN SPIROGYRA WEBERI

(WITH ONE FIGURE)

The writer has reported[1] the occurrence of cross-conjugation in *Spirogyra inflata* (Vauch.) Rabh., which was found in material collected in April 1915. In the spring of 1917 another species was collected in cross-conjugation. Glycerine mounts were made and examined. The phenomenon in this case is very similar to that in *S. inflata*. Table I shows the dimensions of *S. inflata* and *S. Weberi* as given by WOLLE and DETONI, also the material collected by the writer in 1915 (which has been identified as *S. inflata*), and that collected in 1917 which corresponds sufficiently with *S. Weberi* to be identified with that species.

TABLE I

	ZYGOTE		ZYGOTE CELL		VEGETATIVE CELL	
	Length	Width	Length	Width	Length	Width
S. inflata*.....	2×W	30–36	Greatly inflated		42–144	14–18
1915 coll.†.....	45	25.9	85	34	99.9	15.6
S. Weberi.*.....	2×W	26–30	Slightly inflated		100–350	18–25
1917 coll.†.....	62.9	29.6	87.2	35.2	101	25.9

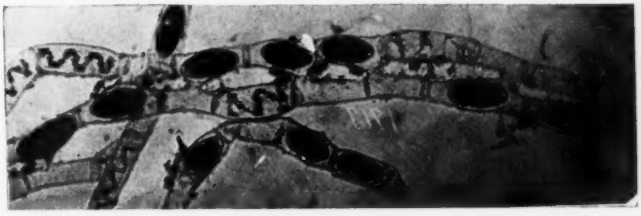
* Dimensions according to WOLLE and DETONI.

† Average of 15 measurements.

Since there is a possibility of considerable variation in the size of a plant owing to various causes, such as food, light, heat, etc., it is probably well to add the fact of the great difference in the inflation of the zygote cells. WOLLE says that the zygote cell of *S. inflata* is greatly inflated, while *S. Weberi* is but slightly inflated. If we establish a ratio by dividing the diameter of the zygote cell (d) by the diameter of the vegetative cell (d'), $\frac{d}{d'}$, and apply it to the material collected in 1915, we get $\frac{d}{d'} = 2.205$; while applied to the 1917 material we get $\frac{d}{d'} = 1.359$. This

[1] CUNNINGHAM, BERT, Sexuality of filament of *Spirogyra*. BOT. GAZ. **63**:486–500. 1917.

shows a remarkable difference between the two collections, and taken with the facts shown in the table leads the writer to identify the 1917 collection as *S. Weberi* Keutz.



a

b

FIG. 1.—*a*, *Spirogyra Weberi; b*, *S. inflata*

These differences are shown in fig. 1, from preparations made at the same magnification, in the same mounting media of identical concentration.—BERT CUNNINGHAM, *Trinity College, Durham, N.C.*

AN ENDEMIC BEGONIA OF HAWAII

The flora of the Hawaiian Archipelago exhibits many pronounced peculiarities. Among these the high endemism, nearly 85 per cent of the spermatophytes, is noteworthy and unexcelled. One of the specific instances of endemism, very interesting to the student of plant distribution, is the solitary begonia, *Hillebrandia sandwicensis* Oliver. This lone species, sharply precinctive in its zonal range, is undoubtedly a vestige of an ancient flora more primitive than that which the islands now possess. Its presence in our flora constitutes one of the many evidences, floral, faunal, and geological, that at one time in the history of the

Pacific Basin the Hawaiian Islands were much more closely associated with the Andean and South Pacific regions than they are at present.

The Begoniaceae comprise 4 genera, of which two are monotypic. *Begonia*, with 400–500 species in tropical and subtropical countries, gives the family its name and definition. *Begoniella* has 3 species in Colombia. *Symbegonia* in New Guinea and *Hillebrandia* in Hawaii are monotypic and little known. As BAILEY[1] remarks, "The begonias are exceedingly variable, the genus running into about 60 well-marked sections, but the intergradations are so many and the essential floral characters so constant that it is impracticable to break up the great group into separate genera."

Considering the family as a whole, it is practically absent from the Pacific region. The two great begonia regions are (1) South America along the Andes to Mexico; and (2) the eastern Himalayas southeastward to the Malay Peninsula. With the exception of the two vestigial and little-known species, one in New Guinea and the other in Hawaii, the entire family is now without representation in the far-scattered island groups of the southern, equatorial, and northern Pacific biological provinces.

The genus and species found in Hawaii was described by OLIVER (Trans. Linn. Soc. **25**:361. *pl. 46*). The generic name is in honor of Hawaii's greatest botanist, WILLIAM HILLEBRAND, who resided in the islands for many years, made an exhaustive study of the land flora, and was the author of *Flora of the Hawaiian Islands* (1888). *Hillebrandia* differs from *Begonia* in having the ovary free in its upper third, and in bearing petaloid organs in the female flowers; in all other features it strongly resembles the true begonias.

This beautiful and interesting plant is confined to the montane rain forest zone. It occurs on all the larger islands of the group, with the exception of Hawaii, from which it has not been recorded. Its altitudinal range is from 3000 to 6000 ft. The islands of Kauai and Maui appear to possess this plant in greatest abundance; it is common in the upper levels of the former, and occurs in practically all of the wet ravines of West Maui and Hale-a-ka-la. In the Koolau Gap of Mount Hale-a-ka-la it attains perfection and a height of 6 ft. On the windward precipices of the island of Molokai it forms a beautiful drapery, and is very showy, although the individual plants are not as fine as those which grow in more sheltered localities. On Oahu it is very rare, and is restricted to the upper levels of Mount Ka-ala, and a few spots in the Punaluu Mountains. It is very shade tolerant and is usually found in

[1] BAILEY, L. H., Standard cyclopedia of horticulture.

the vicinity of waterfalls or in the depths of narrow, sunless ravines. In many of its ecological characters it resembles the endemic *Gunnera petaloidea*.

The native Hawaiian name for *Hillebrandia* is *Pua-maka-nui*, literally "the flower with the big eyes," referring to the large, showy flowers, which contrast strongly with the gloom of its habitat. On the island of Kauai it is known as *Ala-aka-awa;* the Kauai natives use many names and words which are used in no other parts of the islands. The rhizomes of many begonias, particularly those of South America, are bitter and astringent, and are used medicinally by the natives of those countries. It does not appear that the primitive Hawaiians used *Hillebrandia* in any way, although it should be stated that much of the medicinal lore of ancient Hawaii has been irrevocably lost.—VAUGHAN MACCAUGHEY, *College of Hawaii, Honolulu.*

SECONDARY PARASITISM IN PHORADENDRON

BROWN'S[1] illustration of *Phoradendron californicum* parasitic on *P. flavescens*[2] has a twofold interest. First, it records a case of secondary parasitism which seems to be very rare indeed. It has never, so far as I am aware, been noted by workers at the Desert Botanical Laboratory, a number of whom have been especially interested in parasitism. For the most part *P. macrophyllum* and *P. californicum* occur on quite different hosts.[3] Second, the case is of interest physiologically, as BROWN suggests, in its relation to osmotic and other physical phenomena. HARRIS and LAWRENCE, in their study of the sap properties of Jamaican montane rain forest Loranthaceae,[4] find that in these forms the sap extracted from the green stems of the leafless species shows lower osmotic concentration than that from the foliar tissues of the leafy forms. Thus in working with 7 species of Loranthaceae they found average values of the freezing point lowering of 1.153°, 1.176°, and 1.177° in the leafless species as compared with 1.305°, 1.347°, 1.400°, and 1.650° in

[1] BROWN, J. G., Mistletoe vs. mistletoe. BOT. GAZ. **65**:193. *fig. 1.* 1918.

[2] This is presumably *P. macrophyllum* Cockerell, the *P. flavescens macrophyllum* of ENGLEMANN and of some subsequent workers, or one of its varieties. The host here, as Professor BROWN has kindly written me, was a *Fraxinus*.

[3] TRELEASE (The genus *Phoradendron*, p. 14, Urbana. 1916) notes that *P. californicum*, while occurring exclusively on angiosperms, belongs to a group, the "Pauciflorae," which with this and one other exception is limited to coniferous hosts.

[4] HARRIS, J. ARTHUR, and LAWRENCE, J. V., On the osmotic pressure of the tissue fluids of Jamaican Loranthaceae parasitic on various hosts. Amer. Jour. Bot. **3**:438–455. 1916.

the leaves of the leafy forms. If the same is true of desert Loranthaceae, the relationship between leafless and leafy parasite observed by BROWN is just the reverse of what might be expected if successful parasitism were dependent upon higher osmotic concentration in the tissue fluids of the parasite.

As pointed out elsewhere, however, the technical difficulties in the comparison of the tissue fluids of the stems and leaves by the methods as yet available for field work are rather great. In the leafless forms there is danger of including a considerable amount of fluids from woody conducting tissue not at all comparable with that of the green tissue which may be taken to be physiologically homologous with the leaf tissue of the leaves of the tree or of the leafy Loranthaceae. Furthermore, such work as has been done on the rather difficult problem of the physico-chemical properties of the tissue fluids of desert Loranthaceae[5] is insufficient to show that the osmotic concentration is lower in the leafless desert forms. Furthermore, the concentration of the sap of desert forms seems to vary rather widely, and even if the average concentration of the fluids of *P. californicum* were lower than that of *P. macrophyllum*, it is quite possible that the individual secondary parasite, *P. californicum*, had a higher concentration than its individual *P. macrophyllum* host.[6]

So far as I am aware, the only direct determination of osmotic concentration in primary and secondary parasitism in the Loranthaceae is that by HARRIS and LAWRENCE (*loc. cit.*) on the Jamaican broad-leaved *Phthirusa parvifolia* parasitic upon the leafless *Dendrophthora gracilis*, which is in turn parasitic upon a tree, *Cyrilla racemiflora*. The sap properties stand in the following relationship: *Cyrilla racemiflora*, $\Delta = 1.18$, $P = 14.2$; *Dendrophthora gracilis* (on *Cyrilla racemiflora*), $\Delta = 1.26$, $P = 15.2$; *Phthirusa parvifolia* (on *Dendrophthora gracilis*), $\Delta = 1.49$, $P = 17.9$. Osmotic concentration increases from the host to the primary parasite and from the primary parasite to the secondary one. Note also that the observed secondary parasitism is the *leafy P. parvifolia* with an average depression of 1.347° upon the *leafless D. gracilis* with an average depression of 1.176°.—J. ARTHUR HARRIS, *Cold Spring Harbor, N.Y.*

[5] HARRIS, J. ARTHUR, On the osmotic concentration of the tissue fluids of desert Loranthaceae. Mem. Torr. Bot. Club 17:307–315. 1918.

[6] I have individual determinations on *P. californicum* which indicate higher concentration than some found in *P. macrophyllum*. The great difficulty of comparing the sap properties of the two forms lies in the fact that, in the neighborhood of Tucson at least, they occur in the main on different hosts and for the most part in slightly different local habitats.

CURRENT LITERATURE

NOTES FOR STUDENTS

Formation and translocation of carbohydrates in plants.—In a series of three papers from the Rothamsted Experimental Station, DAVIS, DAISH, and SAWYER [1, 2, 3] have reported the results of an investigation designed to test the validity of BROWN and MORRIS' conclusion that cane sugar is the primary photosynthetic product in foliage leaves, that the dextrose and levulose present are products of its hydrolysis, not its precursors, and that levulose is found in excess in the leaves and leaf stalks for the reason that dextrose is more readily utilized in respiration. The introductory review of literature presents an account of work done in this field since the appearance of the memoir by BROWN and MORRIS in 1893. The workers who have given attention to the subject since that time fall into three groups: WENT, STROHMER, STEPHANI, PEKLO, and PARKIN, who adhere to the view that saccharose is the first sugar formed in photosynthesis; MAQUENNE, STRAKOSCH, ROBERTSON, IRVINE and DOBSON, GUTZEIT, and DELEANO, who consider that hexoses are the primary product; and PELLET and COLIN, who hold the belief that saccharose, dextrose, and levulose are formed simultaneously in the leaf and transported as such to the storage organs, where conversion of the reducing sugars into saccharose subsequently occurs.

The authors made analyses of leaves, midribs, and upper and lower halves of petioles of Yellow Globe mangold at three stages of growth: an early stage (August 26) when leaf formation was predominant, the seeds having been sown June 9; an intermediate stage (September 10) when leaf growth had practically ceased and storage of sugar in the root had attained its maximum rate; and a final stage (October 11) when growth of roots had been practically completed. Samples were collected at intervals of 2 hours over a 24 hour period on each

[1] DAVIS, WILLIAM A., DAISH, ARTHUR JOHN, and SAWYER, GEORGE CONWORTH, Studies of the formation and translocation of carbohydrates in plants. I. The carbohydrate of the mangold leaf. Jour. Agric. Sci. 7:255–326. 1916.

[2] DAVIS, WILLIAM A., Studies of the formation and translocation of carbohydrates in plants. II. The dextrose-levulose ratio in the mangold. Jour. Agric. Sci. 7: 327–351. 1916.

[3] DAVIS, WILLIAM A., and SAWYER, GEORGE CONWORTH, Studies of the formation and translocation of carbohydrates in plants. III. The carbohydrates of the leaf and leaf stalks of the potato. The mechanism of degradation of starch in the potato. Jour. Agric. Sci. 7:352–384. 1916.

of the dates given. Chemical changes subsequent to collection were prevented by dropping the material immediately into a large volume of boiling 95 per cent alcohol containing 1 per cent concentrated ammonia, which instantly destroys the enzymes present. In the subsequent analyses, the methods outlined by the authors in their papers on the estimation of carbohydrates in plant material were employed; the chief new features of these methods are the employment of 10 per cent citric acid for the inversion of saccharose, the estimation of maltose by fermentation with maltase-free yeasts, and the determination of starch by the use of taka-diastase.

Maltose and starch were entirely absent from the leaves at all times, day and night, in all three series. Starch is present in very young leaves, but disappears as soon as the roots have grown sufficiently to be capable of storing sugar. In the first series, hexoses began to increase immediately after sunrise, attained a maximum of 2.16 per cent between 10 A.M. and noon, declined sharply until 4 P.M., then decreased steadily throughout the night to start upward again at 4 A.M. The curve representing saccharose rose more slowly from sunrise, maintaining a maximum of 3.11 to 3.06 per cent from noon to 4 P.M., then dropped in an almost straight line through the night to start up at 4 A.M. Both curves roughly paralleled the temperature curve.

In the second series (that of September 10) both curves were complicated; that for hexose shows a minimum at 8 A.M., with a rapid rise to 7.5 per cent at 1 P.M., followed by a slight decline for 3 hours which is succeeded by a rise to 8.9 per cent at 6 P.M. Two hours later this has fallen to 6.75 per cent, but there is again a rise to a new but lower maximum of 7.81 per cent at 2 A.M., after which there is a sharp decline, continuing until sunrise. The curve for saccharose is similar, in that it shows two maxima at 6 P.M. and 2 A.M., but differs in that the second is much the largest, the amounts being 6.39 and 8.27 per cent. The curves for hexose and saccharose in the third series are alike in that each presents three maxima; for hexose these occur at 1 P.M., 9 P.M., and 3 A.M., the last being greatest, while those for saccharose occur at 3 P.M., 9 P.M., and 3 A.M., the second being considerably higher than the others. In neither the second nor the third series is there any resemblance to the temperature curve. In the first series, the amount of saccharose present is at all times much greater than that of hexose, becoming 7 times as great at 4 A.M., and the fluctuations in amount of hexoses are much greater than those of saccharose. In the second series, hexoses vary between 8.9 and 5.4 per cent and are larger in amount than saccharose, which varies from 8.27 to 4.24 per cent. To this statement there is one exception at 2 A.M., at which hour saccharose is slightly in excess. In the third series, hexoses are again in excess, varying from 12.41 to 9.39 per cent, while saccharose ranges between 9.52 and 4.98 per cent. The variations in saccharose are greater than those in dextrose in both second and third series, and are greater in the third than in the second. Consequently, while the ratio of invert sugar to cane sugar varies, in the first series, between 0.133 (at 4 A.M.) and 0.710 (at 10 A.M.), and is expressed by a curve closely

paralleling the temperature curve, the ratio for the second series has a maximum of 1.60 at 4 P.M. and exceeds unity at all hours except at 2 A.M., when it drops to 0.94, but still roughly parallels the temperature curve. In the final series the ratio ranges between 1.93 at 7 A.M. and 1.14 at 3 P.M. as extremes.

For total sugars (hexoses plus saccharoses) the maximum in the August 26 series is 5.26 per cent, reached at 12 noon; the minimum, 1.70 per cent, is attained at 4 A.M.. On September 11 the maximum of 16.08 per cent is attained at 4 A.M., the minimum of 9.98 per cent at 8 A.M., with a second rise to 15.29 per cent at 6 P.M. On October 11 the maximum of 20.99 per cent occurs at 7 P.M., is followed by a slightly lower maximum at 3 A.M., with the minimum, 14.5 per cent, occurring at 7 A.M.

In the first series, pentoses vary during the daylight hours only between 0.37 and 0.45 per cent, dropping again to 0.36 at 8 P.M., only to rise slowly through the night to 0.52 at 4 A.M. Pentosans remain practically constant through the day in the neighborhood of 5.5 per cent, with a maximum of 5.96 at 4 P.M. In the second series, the fluctuations in pentose have much wider limits; there is increase from 0.34 to 0.68 per cent between 10 A.M. and 2 P.M., followed by a fall to 0.45 at sunset and a subsequent rapid rise to 0.71, remaining stationary through the dark hours and dropping suddenly to 0.5 at 4 A.M. Pentosans rise slightly between noon and 2 P.M., then remain stationary until 4 A.M., when there is a second slight rise, but the fluctuations are between 4.42 and 5.9 per cent as extremes. In the final series, pentoses remain almost unchanged at 0.9 per cent from 9 A.M. until 9 P.M., declining to a minimum of 0.61 per cent at sunrise (7 A.M.). Pentosans here show very slight fluctuations, but are slightly higher (6.77 to 7.15 per cent) during darkness than in the day (6.21 to 6.55 per cent). The total percentage of material soluble in alcohol falls slowly throughout the day in the first series, attaining a minimum at 4 P.M., then remains nearly constant through the night. In the second series there is a decline in alcohol-soluble constituents from 4 A.M. to 1 P.M., then a rise continuing until sunset, with a drop between 6 and 8 P.M., then a very slow rise from 47.2 to 51.3 per cent between 8 P.M. and 4 A.M. In the third series the percentages of alcohol-soluble materials are almost constant from 7 A.M. to 9 P.M., varying only from 52.0 to 54.9 per cent, then run down at 11 P.M. to 47.9 per cent, only to return at the next sampling to the general level.

The increases in pentosans throughout the day in the first and second series are attributed in part to the building of new ligneous tissue, in part to the formation of gums which play the rôle of reserves. In the third series, when the leaves have ceased to grow, the fluctuations are apparent rather than real, being due to fluctuations in total sugars. The striking feature of the curves for sugar are the two night maxima which occur in both second and third series, since both hexoses and saccharose increase synchronously to a point higher than that reached during insolation, their sum total also exceeding that attained in the day and reaching its greatest amount at the same time, between midnight and 3 A.M, in both series, so that the results cannot be due to interconversion.

In the entire absence of both maltose and starch, the authors attribute this increase to the conversion into saccharose and invert sugar of some gummy substance, which is present in large amounts and which is precipitated in semi-crystalline form by basic lead acetate after treatment with taka-diastase. There is also an interconnection between the fall and rise of pentoses which occurs between 4 and 8 P.M., and the change in the opposite direction in saccharose and hexoses.

While pentosans make up 8.58 to 9.61 per cent of the insoluble matter of the leaves in the first series, there is an increase to 9.83–10.85 per cent in the second and a further increase to 13.70–15.35 in the third. While in the first series about one-half the saccharose and nearly all the hexoses are used up during the night, the second and third series show a very much larger amount of reducing sugars present at the beginning of the day, and the amount of these up to the attainment of the first maximum is always greater than that of saccharose, but when root growth is nearly complete, as in the third series, the range of variation in cane sugar in the leaf becomes much greater, the leaf apparently acting as a storage reservoir during insolation. The range of variations during growth is summarized as follows:

Series and date	Insoluble in alcohol	Saccharose	Hexoses
I, August 26.....	57.5–62.9	1.50–3.11	0.20– 2.16
II, September 10....	45.3	4.24–8.27	5.38– 8.90
III, October 11	55.8	4.98–9.52	9.39–12.41

	Pentose	Pentosans	Ratio, invert to saccharose	Total saccharose plus hexose
I, August 26.....	0.36–0.52	5.19–5.96	0.13– .71	1.70– 5.20
II, September 10....	0.34–0.76	4.42–5.90	0.94–1.60	9.98–16.08
III, October 11	0.61–0.92	6.21–7.15	1.14–1.93	14.50–20.99

Information as to the translocation of the sugars was obtained by making separate analyses of the midribs and petioles. At any given picking the amount of sugars and alcohol soluble matter is always greater in midribs than in leaves, greater in top halves of petioles than in midribs, and greater in lower halves than in top halves of petioles. In the first series, the total amount of hexoses and of apparent levulose in the stalks increases very rapidly during the forenoon to reach a maximum at noon, while the corresponding increases in dextrose and saccharose are extremely slight, dextrose being actually larger in amount at midnight than at any time during the day. In the bottom halves of the petioles of the series, total hexose, apparent dextrose, and apparent levulose run very closely together, reaching a maximum at noon, declining steadily to midnight, and then separating, as levulose continues to decline while the others start upward again. Saccharose rises slightly from 6 A.M. to noon, and then remains stationary for the succeeding 18 hours. In the second series,

in which top and bottom halves of petioles were not analyzed separately, saccharose was constant throughout the 24 hours; total hexoses and apparent levulose were least at 11 P.M., increased slowly to 4 A.M., then more rapidly until 4 P.M., when they again declined together until 11 P.M. Apparent dextrose rose from 4 A.M. until 4 P.M., then remained stationary throughout afternoon and night. In the midribs, however, total hexoses decreased slowly from 10 A.M. to 4 P.M., then more rapidly through the night, rising again at 4 A.M. Apparent levulose decreased, apparent dextrose increased, from 10 A.M. to 4 P.M., after which dextrose rather rapidly fell off while levulose slowly increased until 4 A.M., when both began to increase. Saccharose was stationary from 10 A.M. to 4 P.M., then increased slowly and uniformly through the evening and night, beginning to fall at 4 A.M. There is, therefore, a steady movement of sugars from leaves to midribs, thence through the stalks, the maximum in leaves at 2 A.M. moving onward into the stalks to give a maximum there at 6 A.M., which is succeeded by a minimum 4 hours later, when a large part of the sugar formed during the insolation of the preceding day has passed from stalk to root. The ratios of invert sugar to cane sugar at any given hour of the day, as at 6 A.M., September 10, when it is 1.48 in leaf, 3.32 in midrib, and 5.27 in stalk, are significant, showing as they do that there are progressively more and more hexoses in the stream of sugars as it passes from leaf to root. On August 26 the stalks had at noon 4.25 per cent saccharose and 11.57 per cent hexose; at 10 A.M., September 10, 4.82 per cent saccharose and 20.5 per cent hexose; and at 11 A.M., October 11, 5.29 per cent saccharose and 25.7 per cent hexose. This is strong evidence that hexoses are translocation forms produced by the conversion of cane sugar, as is the fact that cane sugar greatly predominates in the leaves in the early stages of growth, prior to the beginning of storage in the roots. Further evidence is seen in the fact that cane sugar is the predominant sugar in the leaves of the potato, vine, and snowdrop, although these plants store carbohydrate as starch, dextrose, and inulin respectively, and do not store cane sugar. Cane sugar is therefore formed in the mesophyll, transported into the vessels, undergoes progressive inversion as it passes onward through midribs and stalks, enters the roots as reducing sugars, and these are there transformed once more into saccharose. The authors have not studied the mechanism of this synthesis in the root; invertase was shown to be present in the sieve tubes but was not found in roots by ROBERTSON, IRVINE, and DOBSON, and it is believed to be the agent in the inversion occurring during transport. Since the existence of the saccharogenic enzyme of BORDET has not yet been substantiated, and the probability of reversible zymohydrolysis by invertase is contra-indicated by the absence of invertase from the roots, the authors are unable to formulate a theory as to the agent responsible for this synthesis.

In the second paper of the series DAVIS reports the result of a study of the dextrose-levulose ratio in the mangold. The determination of these sugars by polarimetric methods is falsified by the presence of optically active substances not precipitable by basic lead acetate. Glutamine, glutaminic acid,

and aspartic acid give a dextro-rotation which is increased by acids, while asparagine gives either dextro- or laevo-rotation accordingly as the solution is or is not acid. In the mangold and sugar beet there is an apparent excess of dextrose over levulose which is due to the presence of glutamine, while in snowdrop, tropaeolum, and potato the presence of asparagine results in an apparent excess of levulose. In the first case the apparent excess of dextrose increases progressively from leaves through midribs and stalks as a consequence of the transfer of the impurity; in the leaves the dextrose-levulose ratio is in the neighborhood of unity; in the midribs and stalks it ranges from 2.5 to 10.0. The pentoses which are present in the alcoholic extract also affect the readings.

The author determined the proportions of the two sugars present in the three series discussed in the preceding paper, using the methods there employed. In the early morning there was found in young leaves a dextro-rotation still greater than that which would be observed if all the sugar present were dextrose. In older leaves there appeared to be a steady formation of a laevo-rotatory substance and a gradual transformation into a compound having still greater laevo-rotation. The author considers that this is manufacture of asparagine and transformation into aspartic acid. Both in the second and the third series there are three rises and three falls in the amount of apparent dextrose in 24 hours, this fact pointing to a regular and rhythmical variation in the rate of production of the optically active impurities.

The character of the optically active impurities in the upper portions of the stalks is quite different from that in the lower portions, as shown by the fact that when determinations of the sugars in the lower portions of the stalks are made simultaneously by polarization and reduction methods the polarization results are 40 per cent higher than those obtained by reduction, while on the upper portion of the same stalks the results by polarization are 85 per cent lower than the reduction figures. Hence the optically active substances interfering with the polarization are quite different in the two portions of the stalk, suggesting the optical behavior of d- and l-asparagine and d- and l-glutamine. Furthermore, there are two different optically active substances at different times during the 24 hours. For all these reasons we can at present obtain no true values for these sugars, and there is at least nothing to disprove the assumption that dextrose and levulose exist in the leaves and stalks as invert sugar, travel in approximately equal amounts to the roots, and there undergo recombination into saccharose.

In the third paper of the series DAVIS and SAWYER have applied similar methods of study to the potato as a typical plant forming starch in the leaves, paying especial attention to the mechanism of degradation of starch in the leaf, and have extended the study to a considerable number of plants, including turnip, sunflower, dahlia, carrot, grape, and others. They were able to find no maltose at any time, either during day or night, in plants storing much starch in the leaves, although more than 500 analyses by means of maltase-free yeasts were made. Hence the authors consider that BROWN and MORRIS were

incorrect in their conclusion that diastatic formation of maltose and transfer as such occurs in the case of the leaf of *Tropaeolum*. BROWN and MORRIS unquestionably had maltose present, as shown by the fact that they obtained the osazone and that there was an increase in the reduction of copper after treatment with maltase, but the authors consider that this result may be explained by the fact that the material used by BROWN and MORRIS was subjected to preliminary drying in an oven. They regard the leaf as having a mixture of enzymes analogous to that found in *Aspergillus oryzae*, and that it is therefore able to split maltose rapidly and completely to dextrose. They destroyed all enzymes instantly by dropping the leaves as they were picked into a mixture of boiling alcohol and ammonia. As maltase is easily destroyed by heating to 55°, it was first to go out of action in BROWN and MORRIS' oven-dried material, while other more heat-resistant enzymes went on forming maltose which was not split up, hence was found in the analysis. This hypothesis is borne out by the results; DAVIS and SAWYER invariably found more starch in the leaves than did BROWN and MORRIS, the amount always exceeding the sum of starch plus maltose found by the last-named authors. KLUYVER employed a biochemical method, using *Torula monosa* to ferment the hexoses only, *T. dattilla* to ferment the cane sugar and hexoses, leaving maltose, and found very small amounts of maltose.

The authors consequently believe that starch degradation goes immediately down to hexoses with no stop at maltose; that plants must reduce sugars to this form before they can be utilized; and that the fact that the sugar in leaf stalks is largely hexose is thus explained, as is the presence of invertase in almost all plant parts. DAISH found maltase wherever starch is found in leaves, but believes it to be an intracellular enzyme occurring in close proximity to diastase, hence never found in the vessels of the stalks.

In the leaf saccharose is always greatly in excess of hexoses; in the stalks the reverse is always true. Hence saccharose must be the first product of photosynthesis and hexose a translocation form. The authors are led by unpublished work with a variety of other plants, such as sunflower, grape, and snowdrop, to the conclusion that this is the general situation with all plants regardless of the form in which final storage may occur. Like the potato, the plants just mentioned have two optically active impurities which are formed at different periods in the 24 hours, and hence have apparent large fluctuations in the dextrose-levulose ratio, which it is impossible to measure correctly by reason of their presence.

The authors found in the leaves considerable amounts of dextro-rotatory, water-soluble material which was not soluble starch or dextrin, which was greatest in amount between 4 and 8 P.M. Its period of greatest formation synchronizes with the high tide of saccharose and the period of most rapid starch formation, hence it seems to be intermediate between the hexoses and true starch. Starch is very rapidly reduced after sunset, then more slowly with the hexoses rising, while the starch rises again at dawn considerably before

the hexoses show increase. The curves for hexose, starch, and this dextro-rotatory material are intimately related and indicate interconvertibility; the last-named substance may be a protein or a gum standing in causal relation to starch synthesis.

In the leaves the daily fluctuations of alcohol-soluble substances is through a range almost twice as great as that of total sugars. In the stalks the same is true, in which respect the potato is unlike the mangold. The dextrose-levulose ratio determinations are of little significance because of the presence of laevo-rotatory non-sugars, probably asparagine, but the authors regard them as being present in equal amounts as splitting products of saccharose. The polarization readings for saccharose were aberrant as in the mangold, by reason of the presence of impurities of the same character. Levulose apparently predominates in the leaves and dextrose in the stalks, by reason of the accumulation of dextro-rotatory stuffs in the latter, or possibly by reason of an actual excess due to the using up of levulose in tissue building. That this latter alternative is the correct one is indicated by the fact that the determinations of cane sugar by polarization and by reduction are in close agreement.—JOSEPH S. CALDWELL.

The Oenothera situation.—Three recent papers have cast some light on the perplexing *Oenothera* situation. One of the most serious objections to the mutation theory has been that mutants which have appeared under observation in artificial cultures have regularly been interfertile, while incipient species in nature are essentially intersterile. METZ and BRIDGES[4] have shown that mutants may be intersterile, describing two cases in *Drosophila*, each involving two mutants that either refuse to cross or else give sterile hybrids.

MULLER[5] has explained a curious case in *Drosophila*, which strikingly resembles the *Oenothera* situation. A certain race of *Drosophila* breeds practically true, and yet it is in a heterozygous condition. This paradox is explained by "balanced lethal factors," a given chromosome and its allelomorph each carrying lethal factors. When one of these factors is present in a zygote it brings death, but when both factors are present they are antagonistic in their action and the zygote develops into a mature individual. Thus the homozygotes, which are thrown off every generation, die in infancy, since they contain single lethal factors; only the heterozygotes survive, for in them alone are the lethal factors balanced and inactive. The result is that the heterozygous race seems to breed true. This balanced race, as we should expect, gives in crosses twin hybrids as in *Oenothera* crosses, while crossing two such balanced races in *Drosophila* gives multiple hybrids, as also occurs in *Oenothera*.

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[4] METZ, C. W., and BRIDGES, C. B., Incompatibility of mutant races in *Drosophila*. Proc. Nat. Acad. Sci. 3:673–678. 1917.

[5] MULLER, HERMANN J., An *Oenothera*-like case in *Drosophila*. Proc. Nat. Acad. Sci. 3:619–626. 1917.

Another similarity with the *Oenothera* situation is that in this *Drosophila* race there would occasionally appear recessive mutants on one of these two "lethal chromosomes." These recessive mutants, however, could not become manifest on account of the enforced heterozygosity. They could only become manifest when crossing over occurred and homozygosity was thus made possible. "As crossing over occurs with predictable frequencies, those individuals showing characters abnormal to the stock were thrown continually in a definite, very small percentage of cases." In just such a regular, although small, percentage of cases does *Oenothera Lamarckiana* throw its mutants. MULLER concludes that the *Oenothera* situation is to be explained by a similar mechanism, "but probably the lethal effect in *Oenothera* is on the gametes rather than on the zygote."

A similar idea appears in a paper by DAVIS,[6] in which we find summarized some of the evidence, old and new, on the suspected hybrid condition of *Oenothera Lamarckiana*. The regularity with which the same old mutants are thrown and the production of twin hybrids in crosses suggest to this author the hybrid condition of *O. Lamarckiana*. The facts that about one-half of both pollen and ovules, in random distribution, are sterile, and that only 30–40 per cent of the seeds produced are fertile, suggest that only such gametes and zygotes are fertile as will reproduce the hybrid type. The argument is essentially similar to that of MULLER. "If it could be shown that in every group of 4 pollen grains (tetrad) formed as the result of the reduction mitoses only 2 grains are perfect, the conclusion would be justified that pollen sterility was the result of this segregation division." The author regards this as impossible, however, since abortion takes place after the tetrads have lost their identity. On this point we may quote from a review which appeared in this journal[7] on some work of GEERTS. "In *Oenothera Lamarckiana* 50 per cent of the ovules are found to degenerate and about 50 per cent of the pollen grains, *two from each tetrad of spores*."

It begins to look more and more probable that our classic illustration of mutation is really about the most unfavorable material that could have been chosen for the subject, owing to its germinal complexities. This complexity and seeming lack of conformity have served to make "*Oenothera* genetics" a science in itself. Geneticists will feel relieved when these data on *Oenothera* are finally interpreted by the Mendelian system, and there is now much hope that this may soon come to pass.—MERLE C. COULTER.

Edible and poisonous mushrooms.—Popular interest in the fleshy fungi appears to be growing in many sections of the country. This interest may be attributed to several different causes, chief of which are to be found in the

[6] DAVIS, B. M., A criticism of the evidence for the mutation theory of DE VRIES from the behavior of species of *Oenothera* in crosses and in selfed lines. Proc. Nat. Acad. Sci. 3:704–710. 1917.

[7] BOT. GAZ. 47:481. 1909.

availability of these plants as subjects for nature study and in the desire to add to the dietary a wholesome and palatable food growing without cultivation in forest and field. Here it is almost totally wasted as an article of food, because the comparatively small number of poisonous species cannot be distinguished from the many edible ones, for lack of the elementary knowledge necessary to recognize the more common forms. For this reason, and particularly at this time when there is a worthy desire to conserve every available item of food, nutritious or appetizing, it is gratifying that public institutions devoted to research and to the dissemination of useful information are recognizing the growing demand for popular instruction in the identification of edible and poisonous mushrooms.

One of the most recent pamphlets devoted to this subject is from the Illinois State Laboratory of Natural History.[8] There is an introductory chapter treating in a simple and clear manner of the nature, structure, life-history, ecology, etc., of the fleshy fungi, with a few suggestions as to their collection and preparation for the table. Between 50 and 60 species are described and illustrated by photographs. The arrangement of descriptive text and illustrations is such as to make the work very convenient for practical use by the amateur, and it is to be hoped that the effort of the author will succeed in still further stimulating interest in this group of fungi, often despised under the name of "musheroons," and in leading its users to the desired knowledge of a satisfactory number of edible and poisonous kinds. Following the introductory chapter, two pages generally are devoted to a single species, one page to the descriptive text, and the opposite page to the photograph. As one reads the text the eye easily turns to the photograph in which most of the specific features can be verified.

The photographs are in general good, for many of the specific as well as the generic characters are brought out in detail. To the reviewer, however, they seem to lack the finish and excellence which should be obtained from these plants. Whether this is due in all cases to a lack of care in the original photographs, or to faulty reproduction, is uncertain. The background in a number of cases is unnecessarily spotted, and in general the photographs appear "flat" and not well shaded. It is perhaps a matter of taste in which there may be reasonable differences of opinion, but it would appear preferable that the scale in the photograph should not occupy such an obtrusive position as it does in covering up parts of the plants, when it would serve as good a purpose if placed by the side.

It appears that a few of the plants are not correctly named. For example, pl. 137 does not appear to be *Clavaria cristata;* pl. 119 is probably all *Craterellus cantharellus;* pl. 113 does not resemble *Pholiota squarrosa*, but rather a *Hypholoma*, related to or identical with *H. lachrymabundum*. The omission of *Amanita "muscaria"*, a very poisonous species of wide distribution, should be noted. —Geo. F. Atkinson.

[8] McDougall, W. B., Some edible and poisonous mushrooms. Ill. State Lab. Nat. Hist. Bull. 11:413–551. *pls. 85–143.* 1917.

Economic importance of diatoms.—MANN[9] gives an interesting discussion of the uses of diatoms. Among these he enumerates the use of fossil diatoms as abrasives in polishing powders, tooth powders, etc. They have also been used as a food adulterant by mixing with flour, thus increasing the bulk of food but adding nothing to its nutritive value. They were used in this way by the "Earth Eaters." A later similar use was as an adulterant of candy, but this use is now prohibited by law. They were also formerly used as an absorbent of nitro-glycerine in the manufacture of dynamite. There are beds of diatomite several hundred feet thick on the Pacific coast, and the use of them as a substitute for asbestos in packing steam pipes, as filler for refrigerators, and in the manufacture of pottery is increasing. Another new use in medicine is as a filter for serums. It is suggested that their beautiful designs be used as patterns in the ornamentation of jewelry, wall paper, etc.

Since diatoms store their food in the form of oil instead of starch, it is believed that they have been one of the sources of petroleum. On account of their being so minute that living ones may be carried great distances in the ocean, they may be of use in determining the direction of ocean currents. One argument that supports NANSEN's theory that there is a current passing northward from Behring Strait across the north polar regions and down the coast of Greenland and Norway is that the diatoms of these localities are of similar species. Perhaps the one use that is of supreme importance is the furnishing of food either directly or indirectly for aquatic animals. Diatoms are chlorophyll-bearing plants, and are the greatest agency in the water for changing inorganic into organic matter, hence a knowledge of diatoms is fundamental to a study of the food supply of fish and other aquatic animals. Animal life is very abundant on the shores of the Antarctic continent, and in that region there is very little land vegetation. The greater part of the food for all of these animals is supplied originally by the diatoms.

The statement that EHRENBERG estimated the number of individuals in a cubic inch of diatomite at 40,000,000 should be 40,000,000,000. The statement is made that diatoms are so minute that 100 of them could be placed on the head of a pin. This is well within the facts, for that number of the smallest could find room on the point of a pin. The use mentioned of the diatoms *Pleurosigma angulatum* and *Amphipleura pellucida* as test objects for microscope objectives has been discontinued. The Bausch and Lomb Company state that the "Abbe test plate" is now used entirely and is more accurate and reliable.—C. J. ELMORE.

Addisonia.—The second number of the second volume of this finely illustrated series, issued June 30, contains colored plates and popular descriptions of *Solidago juncea*, *Echeveria multicaulis*, *Catasetum viridiflavum*, *Sagittaria latifolia*, *Baccharis halimifolia*, *Xanthisma texanum*, *Secum Bourgaei*, *Cimicifuga simplex*, *Feijoa Sellowianus*, and *Aster amethystinus*.

[9] MANN, ALBERT, The economic importance of the diatoms. Smiths. Rep. 1916: 377–386. *pls. 1–3.* 1917.

The first number of the third volume of this journal, published by the New York Botanical Garden, contains colored plates and popular descriptions of *Anonia atropurpurea* (Eastern North America), *Aster novaeangliae* (United States and Canada), *Gymnocalycium multiflorum* and *G. Mostii* (Argentina), *Euonymus alata* (Eastern Asia), *Diospyros virginiana* (Eastern United States), *Lepadena marginata* (Central and Western United States), *Maackia amurensis Buergeri* (Japan), *Hibiscus oculiroscus* (Eastern United States), *Cornus officinalis* (Japan), *Opuntia lasiacantha* (Mexico).—J. M. C.

Morphology of wheat.—JENSEN[10] has investigated certain strains of wheat and the result is perhaps our fullest account of the morphology of this important plant. The subjects considered are development of spike and flower, of microspore and male gametophyte, of megaspore and female gemetophyte, fertilization and development of embryo, and endosperm. An interesting record is that fertilization occurred from 32 to 40 hours after pollination.—J. M. C.

Intrafascicular cambium in monocotyledons.—Mrs. ARBER[11] has added to her previous observations[12] of intrafascicular cambium in monocotyledons other observations which include Araceae, Dioscoreaceae, Iridaceae, and Potamogetonaceae. Such cambium is now known to occur in "all but two of the cohorts into which ENGLER divides the monocotyledons; the exceptions are the Triuridales and the Synanthae."—J. M. C.

Seed position and growth.—It has been found that bean seeds planted with the eye up give a somewhat lower degree of germination and growth than when the seed lies flat or is placed eye down.[13] This seems to show that the common practice of dropping seeds flat upon the soil when planting gives results that are satisfactory.—GEO. D. FULLER.

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[10] JENSEN, G. H., Studies on the morphology of wheat. Bull. 150, State Coll. Washington. pp. 21. *pls.* 5. 1918.

[11] ARBER, AGNES, Further notes on intrafascicular cambium in monocotyledons. Ann. Botany **32**:87–89. *figs.* 4. 1918.

[12] BOT. GAZ. **64**:350. 1917.

[13] HALSTED, B. D., and OWEN, E. J., Environment of seeds and crop production. Plant World **20**:294–297. 1917.